

State of Kuwait
Series of Publications of .
Islamic Organization For Medical Sciences
Islam and Recent Medical Problems

Phytochemical And Biological Evaluation

of

Some Plants Used in Islamic Medicine

(52)

Supervised by
Dr. Abdul Rahman A.Al-Awadi,
President,
Islamic Organization
for Medical Sciences, (IOMS)
Kuwait

Edited by
Dr. Ahmad Rajai El-Gindy,
Secretary General Assistant, IOMS
Dr. Mokhtar M. Bishr
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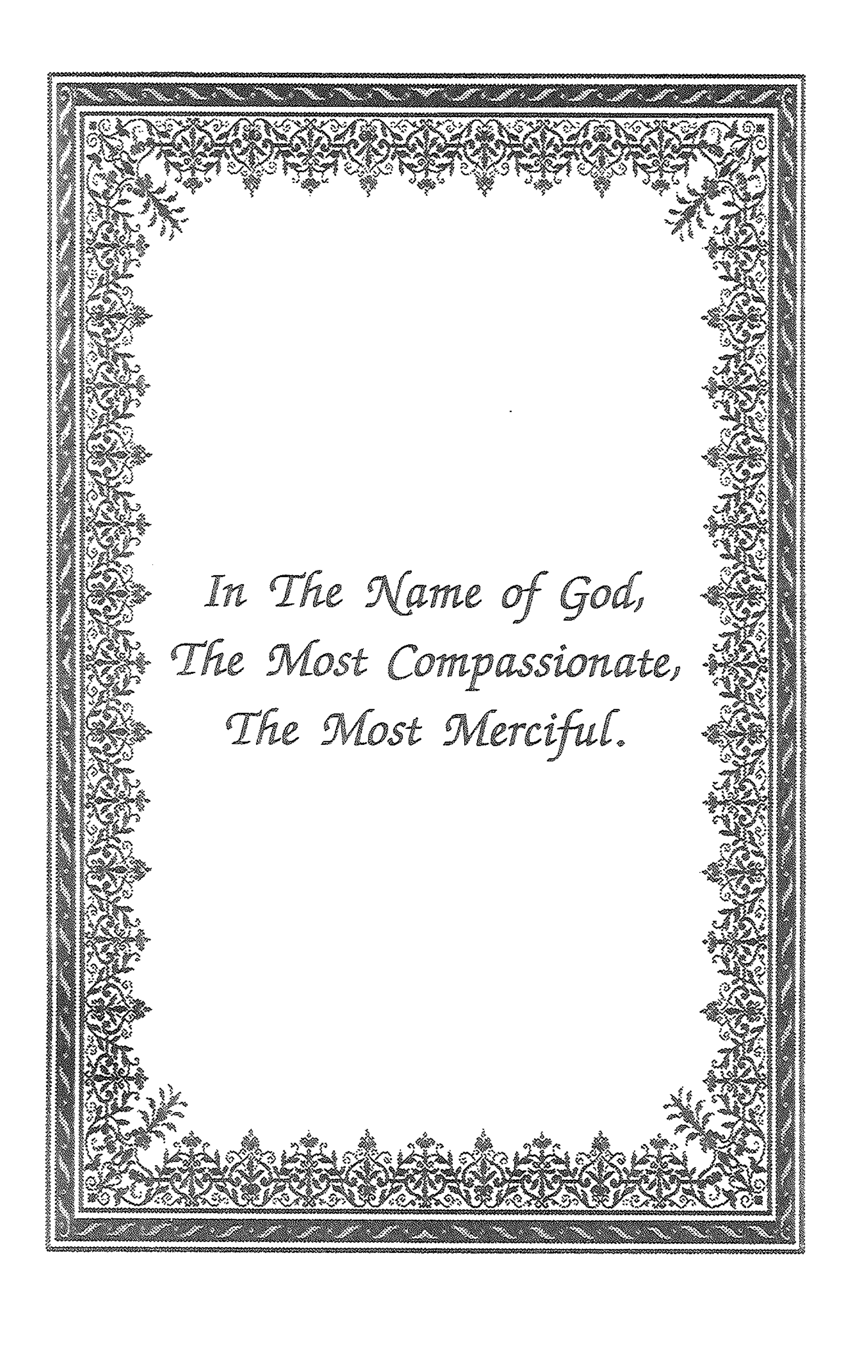
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In The Name of God,
The Most Compassionate,
The Most Merciful.

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PREFACE

Dr. Abdul-Rahman A. Al-Awadi
President
Islamic Organization for Medical Sciences
KUWAIT

Thanks to Allah Almighty for guiding us to Islam, enlightening our hearts with true belief, discarding all grief, dispelled worries, and freed our homeland.

This series comes following fifteen years of the idea of establishing the Islamic Organization for Medical Sciences and after its participation in local and regional book exhibitions where our volumes of Islamic Medicine were greatly appreciated by the visitors. However, because of the soaring cost of paper and publication, the individual book keeping has become very difficult, especially in the non-Gulf Arabic and Islamic countries, as bread earning receives the first priority of the inhabitants of these countries. Keeping in view the fact that the individuals need to be informed, and educated, of the important matter to make them effective member of their community and also a messenger to other communities, it is vital to provide them the contents of these conferences in a simplified way to enable them to carry along and comprehend the scientific purport.

In order to facilitate the possession of these books by the individuals, the Islamic Organization for Medical Sciences has decided to issue a series of publications under the title "The Cultural Series of the Islamic Organization for Medical Sciences". Although the Organization is shouldering the largest share of the cost of production and publication of these books, still these are out of reach of a large section of Muslim individuals, due to escalating

cost of living. The great sum of money available to the Organization is spent in bringing together and collecting the prominent thinkers of our Islamic nation in order to achieve appropriate opinions and covisions of the Islamic Scientists about right topics that need insight and the true objective word. And, subsequently, to present this information to every individual willing to increase his/her knowledge about the doctrinal writings in scientific medicine, as this prominent group of writers/thinkers sees this as an ordinance and a religious obligation to provide for all the Muslims, and to disseminate the message to the largest number of the people of this nation.

This series will include a group of books, each dealing with specific topic, as collected from the articles written under the respective domains and previously published in the Proceedings of the Islamic Medicine Conferences held under the auspices of the Organization. Moreover, all these publications shall remain concerned with one vital topic, that is, the Islamic Medicine. By doing so, we hope to have shouldered the burden off the Arabic/ Islamic reader to enable him/her to own the right material and hoping to have clarified a lot of mystery about the subject of Islamic Medicine to the Muslim and Arab readers.

Herein, I beseech Allah to guide our steps to what He likes and approves of.

INTRODUCTION

Dr. Ahmad Rajai El-Gindy
Secretary General Assistant
Islamic Organization for Medical Sciences,
KUWAIT

Thanks to Allah, the Almighty; the thanks of the grateful, the obedient, and the desirous of His forgiveness and retribution, beseeching him, to guide us to the right deeds, with praying and blessing his illiterate prophet (ﷺ) who said,

"When Adam's son dies, everything is separated from him except for three things, a current charitable deed, a righteous boy praying for him, and a useful science."

We pray to Allah that these series of publications will be of scientific use to the Muslims in particular, and to humanity in general.

This introduction will be included in all the publications of this series in order to acquaint the reader, who wishes to acquire one or more parts of it, with the objectives of the Organization, and the reasons behind its being established. We wanted to put down these words to the readers concerned about what we did, while the second part of this introduction will be specifically written for each book, including a summary of the researches therein.

Since the emergence of the idea of the Islamic Medicine fifteen years ago, the discussion of the meaning of "ISLAMIC MEDICINE" did not stop; the people argued: Is there an "Islamic" and "a non-Islamic" Medicine? and we found ourselves in front of three opinions:-

The first opinion:-Medicine is a human heritage; inherited successively by generations, and it is a human experience, acquired by technical and scientific practice, and religion has no role in it,

and there is no need to indulge Islam in this subject to protect it from human practices.

The second opinion:- Islamic medicine means nothing to them except it is a past heritage, and we do not need it now because the world is talking about organs transplantation, genetic engineering, Lazer beams... etc. They even considered it a call of underdevelopment, and we have to put it behind closed doors; those are who don't want Islam to be mentioned at all.

The third opinion:- Although medicine is human practices and experiences, but every religion and every heavenly message has its own nature, ethics and practices which are derived from its teachings, and which adds to it its own style. The Islamic era was characterized with a comprehensive change in both the concepts and practices of the people; these concepts and practices were derived from the Holy Quran and the honored Sunna, and were followed by the Orthodox Caliphs, which produced a good harvest, with which they ruled the world, east and west with a civilization - Man was its master, good science its way and the strong belief its pillars. This civilization lasted for five complete centuries, and it was never stingy with its knowledge and arts on humanity.

For there is no favor of an Arab on a Persian, nor of a white man on a black man except by piety and good deeds, this was said by the enemies before the friends; and (Sarton's) testimony in his encyclopedia, the history of sciences, is the best evidence; (Sarton) divided the world into eras of civilizations like the Pharonic, the Babylonian, the Somarian, the Chinese, the Greek, then the Islamic Civilization which flourished in all walks of Arts and Sciences for five consecutive centuries, and in it were eminent scientists, thinkers, philosophers, physicians, pharmacists, engineers, Algebra's, Astronomers, Agriculturists, and people of thoughts who were distinguished with their excellence in the Divine Law, besides the cosmetic sciences.

To all these we say, our view of this topic is derived from Islamic Law, which came with its five goals, which are sustaining the religion, the mind, the self, the honor and the wealth. If we studied these goals, we'll find that three of them are concerned with Man's well being; that is the mind, the self and the honor, as for the other two, they are concerned with man's health, as there is no keeping of religion, nor of wealth without a strong good Muslim (The best one to hire is the strong and honorable). The prophet (ﷺ) defined three main points, if provided in any MAN, he will lead a very happy life, as he (ﷺ) says

"The one who sleeps secured in his bed, healthy in body, well provided for his day's food, ... he is like the one who owned the entire world."

In other words, he has got social, health and psychological security. Thus the Islamic Law talks about well being in its widest range. "The strong believer is more loved by Allah than the weak one, and both are good." The Islamic Law did not speak about medicine in its narrow sense, through which the others are trying to attack us, but medicine is the means of health, and Al-Ghazaly, a Muslim religious leader, considered medicine as a religious ordinance in all Muslim homes.

Islam considers enjoying a good health one of the biggest blessings of Allah; as mentioned in the wise saying of the prophet (ﷺ), "*Two blessings many people are not endowed with; health and leisure time*". These two blessings are two of the very important duties that must be kept by man as the Islamic rule says, "Whatever is not perfect without a duty, is itself a duty", thus man is not allowed to neglect his health, as it should not be neglected, because this is considered an aggression on the whole nation as it is so mentioned in the Holy Quran:-

"FOR THAT ACCOUNT WE ORDAINED FOR THE CHILDREN OF ISRAEL THAT IF ANY ONE SLEW A PERSON -

UNLESS IT BE FOR MURDER OR FOR SPREADING MISCHIEF IN THE LAND - IT WOULD BE AS IF HE SLEW THE WHOLE PEOPLE, AND IF ANY ONE SAVED A LIFE, IT WOULD BE AS IF HE SAVED THE LIFE OF THE WHOLE PEOPLE"

(Al-Maeeda: 32).

Abu-Bakr, (رضي الله عنه) said "I heard the prophet of Allah (ﷺ), saying, *"Ask Allah for certainty and health, for they are the best blessings bestowed on man is being healthy after being certain"*; thus self-relief is the true gate to health; either psychological or bodily health, their only true gate is strong belief, belief in slavery to Allah, whatever inflicts you was not to wrong you, and whatever to wrong you, was not to inflict you.

The belief in the acts of worship which are prescribed by Islam are:-

Prayer is secret talk with Allah Almighty, and self purification five times a day standing in front of the Creator,

Fasting is self restrain from evil desires, and true feeling of the hunger of the Muslim brother who is deprived of a morsel of bread,

Zakat or *Alms* is a sacrifice, self cleanliness, and development,

Haj is a migration to Allah and his prophet, (ﷺ), leaving everything - power, wealth, prosperity and living in complete humbleness and slavery, equal with your kin Muslim... as it is said; "No Arabic is better than a non-Arabic, nor a white is better than a black man except by piety", and these acts of worship protects and restrains man from evil doings, thus leaving them will lead to the spread of evil deeds and man will gain nothing but punishment for what he had done.

In order to complete the building of man and society, and to achieve the goals of Islamic Law, the doctrines of lawful and unlawful were put down to guide man to the right road and bestow happiness on him; as in the lawful deeds man will find his happiness, and in unlawful deeds he will be perished; thus the

prohibition of drinking alcoholic drinks, and all ways leading to it, as prescribed by Allah was for the protection of man's mind and body, the society from diseases and the consequences of the absence of his mind, the prohibition of adultery, and all ways leading to it, wanton display of beauty, solitude with a woman, and libertinism... etc, was prescribed to protect the family and the whole society from dissociation and mixing of lineage which destroy the society, thus the philosophy of prohibition in Islam is meant for the prevention of harms to man himself and to others as well.

Thus, it is clear that the goals of Islamic Law (Sharia) can not be achieved without good health and well being, as Abu-Al-Dardaa said to the prophet, (ﷺ), "To be healthy and grateful, is much more better than to be ill and endure patiently", the prophet (ﷺ) answered him by saying, *"Allah loves healthy people, as you do"*.

That is not all, but Islam's view of the sick and sickness has overrun all that preceded it and whatever followed from laws or social systems, as Islam does not see sickness as an anger of Allah, or a touch of the devil, but a trial, and the Muslim has to be patient and bear it with patience as the Prophet (ﷺ) said,

> *"Any kind of sadness or grief or even the prick of a thorn that inflicts man is a blessing from Allah as He raises him a degree higher or takes from his bad deeds instead".*

The Holy Quran came to the world with statements about the inner self, this was fourteen centuries ago, and it put to it four marvelous divisions in various parts of the Holy Quran, thus the world knew about the peaceful innersoul, the lamenting innersoul, and the authoritative innersoul. Abu-Hamid Al-Ghazally, has delved deep in the inner-self in his encyclopedia "The Revival of religious sciences", under the heading" Fear and Request", as the Holy Quran talked about the ailments of the heart, and their different kinds, as it was mentioned by Imam Al-Zahaby in his book "The Prophetic Medicine".

As for the medicine of the heart, it is only found in the sayings of the benevolent and kind Prophet (ﷺ), when he quoted Allah, the only source of all knowledge, he says that for the hearts to be righteous, it must know its creator, His names, characteristics, deeds, orders, and prohibitions and anger, as there is no way of being righteous except by doing this, and no way of getting these advice except from Mohammed (ﷺ).

Imam Ibn Kerium Al-Jozeiah has divided the hearts into two divisions: suspicion and doubt, and desire and error. He quoted the Holy Quran as saying,

"IN THEIR HEARTS IS A DISEASE; AND GOD HAS INCREASED THEIR DISEASE".

(Al-Baqarah: 10), and:

[O CONSORTS OF THE PROPHET! YE ARE NOT LIKE ANY OF THE OTHER WOMEN: IF YE DO FEAR (GOD), BE NOT TOO COMPLAISANT OF SPEECH, LEST ONE IN WHOSE HEART IS A DISEASE SHOULD BE MOVED WITH DESIRE]

(Al-Ahzaab: 32).

The Quran described the inner-self when horrified or frightened, and how to make it peaceful again in His very simple and clear words:

"TRULY MAN WAS CREATED VERY IMPATIENT; FRETFUL WHEN EVIL TOUCHES HIM; AND NIGGARDLY WHEN GOOD REACHES HIM; NOT SO THOSE DEVOTED TO PRAYER: THOSE WHO REMAIN STEADFAST TO THEIR PRAYER; AND THOSE WHOSE WEALTH IS A RECOGNIZED RIGHT FOR THE NEEDY WHO ASKS AND HIM WHO IS DEPRIVED (FOR SOME REASON FROM ASKING) AND THOSE WHO HOLD TO THE TRUTH OF THE DAY OF JUDGMENT; AND THOSE WHO FEAR THE DISPLEASURE OF THEIR LORD, FOR THEIR LORD'S DISPLEASURE IS THE OPPOSITE OF PEACE AND TRANQUILLITY."

[Al-Maarij: 19-28].

This is how Islam considers health, which was defined by the prince of Islamic physicians: Ibn-Sina by saying: "Medicine is the science by which the human body is known, and what is good and what is not for being healthy or otherwise." This comprehensive definition which was introduced more than one thousand years ago, is nowadays adopted by the WHO, that health is the state of the healthy body, mind and society, not only the lack of diseases or inability.

In spite of this definition of the WHO, during the forties, it ignored the spiritual side, which shows the lack of a comprehensive view of Islam about health, as Islam defines health from all domains, bodily, spiritually, psychologically and socially, and this last definition came 14 centuries ago, by the Muslim physicians.

To reach these noble goals, and great objectives for the Lord's heir on earth, there had to be a way to keep man healthy, and this is by the science of medication which was considered by the Muslim religious scientists an ordinance in the Islamic world, and Imam Al-Shafeiy said about it; "There is no knowledge, better than the prohibited, and non-prohibited acts, to my knowledge, except the science of medication". Dawood Al-Antaky in the introduction to his famous prescription says that there is no science that can do without the science of medication, because no acquisition of any knowledge is perfected without a sound body, senses, and mind.

Islam has taken good care of the different branches of medication; protective, preventive, an rehabilitative; in the protective, many sayings of the prophet (ﷺ), called for protection, in order to keep health in all its branches - cleanliness, food organization, and many healthy habits, as well, the researches in this domain is varied and all are derived from the prophet's (ﷺ) wise sayings, no need to repeat them here.

As for the treatment side Islam legalized medication, and the prophet (ﷺ) ordered medication and looking for it when he (ﷺ) said:

"Ye believers, get treatment, the Lord created no disease without its medicine, known to those who know and ignorance to those who don't know".

As for rehabilitation, we are asked to look for it, he allowed one of his disciples to put a piece of gold on his lost nose during his invasions.

As for the three opinions pre-mentioned concerning the definition:-

To the first group we say: Medication is a human heritage and contribution, but the human thinking has deviated from the right path, and religion is in the church and in the mosque or the temple, due to their sufferings from the control of the church over medication and sciences, and making them only for the priests, medication did not develop, and the ship of science sank deep with its arsenal of destruction, thus they produced the microbial bombs, and medication turned into fatal poison; instead of relieving pains, and becoming a tool of the Lord's benevolence, it became devastatingly harmful, and the brother became keen on eliminating his human brother, and the call for killing substituted the call for mercy, the organs began to be sold, and man was transferred from the master of earth to a sample in labs, and source of trade .... etc. the list is endless.

The best evidence to be quoted here is the saying of Abenhaimar; the father of the atomic bomb, when he saw it explode in Hiroshima from a distance, he said his famous words: "Now, and now only, science has sinned".

As for the second group: which said "Islamic medicine is nothing but an ancient memory and a call for underdevelopment.." we say to them that the heritage of any nation is like the roots of a tree, whenever it goes deeper and deeper in history, it becomes firmer and firmer and provides it with the means of living; the invention of genetic engineering, the nuclear bomb, and organ

transplantation are not only signs of civilization, but they are the leaves of the tree and its fruits, as civilization is much more wider than that, and cares less with its achievements, but cares more for the achiever, MAN, and cares for the philosophy of his existence in this world and the hereafter, as well as his ethics and culture.. if he is separated from these, he will be lost for ever. Now although the western man enjoys the highest per capita, and has got every means of prosperity, we find the percentage of suicide going up and up, as well as the addiction of narcotics, drugs... etc. became a daily practice; to enable him to forget and escape from his worries... the western man neglected the spiritual side of feeding his inner-self, and instead tried to feed on earth's food, thus he failed, and was transferred to a cog in a big machine.

This is not only in the west, but it is now prevalent in the east, as well; family relations are severed, social relations collapsed, man changed into a wild beast in a jungle full of fierce animals, each is trying to eat the other. I don't want to say more, it is enough to remind you with the AIDS that is harvesting man's bodies... Nevertheless, no body talks about chastity, virtue or ethics.. but they began to distribute contraceptives, for males and females, as if saying "Do it however, and whenever you want..! but use these contraceptives to protect you from the AIDS..!" Is this the Islamic way or attitude towards the man, whom it honored and asked to walk and learn and enjoy the fruits of life. Man asks, as many asked before about health and happiness, in spite of his materialistic progress and scientific development in all fields of medicine and protective treatments.

Islam gave due attention to man's environment, and warned him against corruption and doing mischief, as both affect his health, the Lord's words describe what happened all over world from corrupting the environment, which threatens man's life as He said' "CORRUPTION HAS APPEARED ON LAND AND IN

SEA ON THE HANDS OF MAN, TO MAKE HIM TASTE SOME OF HIS DOINGS, HOPING HE MIGHT RETURN TO RIGHTEOUSNESS", and He orders us not to do mischief by saying, "DON'T CORRUPT THE EARTH AFTER IT HAS BEEN RECLAIMED." Corruption here, I believe is both materialistic and ethical; as material corruption includes mischief on earth and around it, and ethical corruption means self and moral corruption.

To add to all these views that each civilization has its characteristics, its features, its morals, and its practices, Islam is unique in this, as Islam sees man as a whole, body and soul in full balance, none overweighs the other, as he did not worship the material, nor invented priesthood. Islam has taken care of man before he was born, when choosing a wife or a husband, at marriage, when he was a sperm drop, a baby, young, and old, Islam put to him a very accurate disciple system of life, taught him how to eat, drink, dress, treat himself, his Lord, his family, and his community. Islam has put to him goals in life - as it is a farm for the hereafter, to harvest from what his hands grew, and Islam was able to introduce a civilization to the world, with which Europe progressed from its dark ages with the help of the Islamic doctrines, but the Muslims slackened down and left Europe to lead the ship of scientific development. It may be that our interest in calling medicine by the Islamic Medicine, came as a symbol to awaken the Islamic world, and tell them that there is a lot in Islam in all fields; economic, architect, arts, cosmetic, medical... etc. and their commitment to Islam will bear fruits, too. One objective of choosing this name to medicine is the human deviations in practicing medicine in the West, but the East has to have a loud voice to awaken it and shake it; that is the voice of Islam, by providing the right opinion in these practices, especially when we lost the lead of materialistic science, but we can still provide it with

what purifies them and saves them from deviation, this is by means of the enlightened Islamic views. Moreover, the communication revolution has made the world a small village, knowing what happens all over it by the second... these developments are knocking our doors, thus we must be aware of it and give the Islamic view point in it, showing the advantage of Islam which differentiates between what is right from what is not.

The Lord knows what the inner-self whispers, as He is nearer to him than his vein, and He is the maker of his inner-self, and He directed him to his success, as He says'

"BY THE SOUL, AND THE PROPORTION AND ORDER GIVEN TO IT. AND ITS ENLIGHTENMENT AS TO ITS WRONG AND ITS RIGHT. TRULY HE SUCCEEDS THAT PURIFIES IT, AND HE FAILS THAT CORRUPTS IT".

(Al-Shams: 7).

The Almighty knows what the corrupt eye sees and what is hidden in the hearts.

Some people suggested that we call it THE ARABIC MEDICINE, in order not to distort the picture of Islam, as a result of misdemeanor of some practitioners, but this name might lead to the understanding of the use of medicinal plants and ancient medication practices, and this has its shortages, as well as its advantages, too, and because most of those who enriched the Islamic movement were not from the Arabic environment, like Al-Razy - from Al-Rey, Ibn-Sina - from Russia, and Al-Bukhary - from Tashkand... etc and thus we'll enter into the vertigo of apartheid, but Islam had engulfed them all. Moreover, if we want to discuss the point of view of Islam in modern things, on what ground shall we argue? Are there Arabic foundations? or, all the foundations taken from the Islamic Law (Shareeaa)? Thus the best name was "THE ISLAMIC MEDICINE", which is nearer to the fact, as for the fear of the misbehaviors, which might be alluded to Islam, wrongly, we know

that all Adam's sons are sinners, and the best sinners are the repentants, we are in a stage trying to erase eras of Islamic decay and weakness, we want to contribute to Islam and to be affiliated to it again, as well as to revive its name and face all over the world, and to prove that its doctrines are applicable, and their consequences are guarantee for man's well being and prosperity.

The Organization aims, also, at retrieving the Islamic behavior which was defined to Man by Islam, and make part and parcel of his daily conduct; if cleanliness, for example, is part of the belief, as said by the prophet (ﷺ), we find our Islamic states are the least countries enjoying and abiding by this Islamic ordinance, although it is the main road to health, and there are many wise sayings which organize the life of the Christians as well as the Muslims in order to lead a healthy and clean life, in the same way the orders and prescriptions in Islam are all related to man's psychological, social and body health; like prayer, fasting, Zakat, Haj, and others of the ordinances that have spiritual meanings which invests in Man tranquility and protects him form psychological and body diseases. There are many researches reinforcing these hypotheses, and the things that Islam forbids us from doing are essentially for our sake, we are not far away from what the world is suffering from narcotics, alcoholic drinks and AIDS which Islam prohibited.

We also wanted to utilize the plants which we have as a gift from the Lord, and Muslims have surpassed the world in this field, thus they kept their heritage of plants for the future generations, moreover they added and developed it. They wrote many books from which the Europeans took and translated and utilized till the 19th century; all their experiments and observations built on high scientific standards: Al-Hawy is considered the first scientific clinical encyclopedia in the history of the medical sciences.

Islamic civilization, at that time, was able to open its arms welcoming every active worker, Muslim or non-Muslim, as Islam

has no discrimination, and no coercion in religion, no one is better than the other except by worship and good deeds, thus scientists migrated to it from east and west to add to its sciences.

I'll mention here, only, the testimonies of some Western scientists for the Islamic civilization:- "Froje Garoody" talks with sadness and grief about western Civilization; he said. "The Western civilization is dying and committing suicide because it deviated from following the natural disposition; the instinct, and its masters considered man the director of the nature which he ruled, but after five centuries of the experience we found out that Nature is the main store of the primary materials and the place for man's leftovers, this made us always destroy nature, and this is against what the Holy Quran decided, as it decided that man is the Lord's heir on earth, and man is concerned with keeping natural balance"; then he says; "Our present western civilization is dying, not because it is short of means, but because it lacks goals". Man began to threaten himself with annihilation, and the result is the destructive weapons that man possesses are enough to destroy the planet earth one hundred times, what poor creatures we are!

This civilization is carrying in its womb the causes of its destruction, on the contrary of the Islamic civilization because the Islamic civilization is coming from the Lord who made it, not man, nor is the Islamic civilization an extension of history, but a revelation from the Lord to His prophet (ﷺ) through the Holy Quran, dictating a Holy Constitution satisfying the body and the spiritual needs of the human beings, then following this came the wise sayings of the prophet (ﷺ) to explain the Quranic doctrine, thus everything became clear, the lawful is clear and the unlawful is clear, and the difference between them is clear. The world is about to face a crisis due to its losses from addictions, as the costs of these addictions reached 14 billion $ in one year in the USA only, and these losses were in work hours, accidents, family problems... etc.

due to the addiction of narcotics or alcoholic drinks, which Islam prohibited. This big sum of lost money is more than the revenue of many countries, and the world will face more than 40 million individuals inflicted with AIDS by the year 2000, and 10 million orphans; the WHO estimates the number will be doubled, nevertheless, virtue is absent, chastity killed, and they don't know where they are going... and no body knows!

Max Mayerhoof testifies: "The Islamic medicine has reflected the sun shine which was setting in Greece, and the moon glittered in the sky of the dark ages, and other stars brightened by themselves and lit the gloomy dark sky, then the moon went down and the light of the stars waned in the revival age, but their traces are still there, to be felt in the civilization of today.."

Montgomry Watt said; "I'm not going to look at Muslims as a barbaric army invading Europe, but I'll consider them the representatives of a civilization which achieved great successes all over the world, spread them to their neighbors. The Europeans are not appreciating their debt to the Islamic Civilization!! They even try to find faults with the volume of the Islamic effect and its importance in our cultural heritage, forgetting, again, that our good relations with the Arabs and the other Islamic nations calls upon us to be aware, to the end, that we owe them, not to mention this truth, or its denial is not right..."

Montgomry Watt didn't stop at that, but he added, "Our following the Arabic Medicine, which lasted till the 15th and the 16th centuries is evidently clear in the printed books, and the first of these books was explanations of the 9th chapter of the Principles of Al-Razy, then followed the printing of Ibn-Sina for three times, before Galinos, and till the year 1500 sixteen editions of "Al-Kanoon", the "Law". The statistics show that the quotations and extracts found in the early European writings are evidence that the

impact of the Arabic books surpassed and surmounted the Greek one.

He says, too, "Islam in essence is not only a mere religious movement, but it is also a human value embedded in life of the peoples who embraced Islam, or joined it, it was a kind of unique human existence in the world as the conditions of the Islamic openings were to permit the other people to continue practicing their former habits, laws, and languages, for paying taxes (Jiziah), these Islamic rules strengthened the relations between the Muslims and the peoples of the countries they conquered, thus the people continued to practice sciences, arts and especially medication.

These three testimonies are only a sample, there are a lot of others for which there is no space to quote here, but in time we will.

In addition to this, the last WHO statistics mention that 25-30% of the diseases from which man suffers nowadays are caused by the side effects of the chemical medicines, as well as their high prices, and the expertise which they need to manufacture. Contrarily, however, our Islamic countries enjoy a suitable weather for the medicinal plants to grow and treat a lot of diseases. All we need are issuing political decrees as China and India and other nations which produce these medications in the most modern fashion.

This is a short synopsis about the idea of Islamic Medicine, and to reinforce this idea, we invited a group of Muslim thinkers to take part in many conferences to write in this field, and we have received a lot of their contributions which will be published in due course of time, under different headings.

SUMMARY OF THE RESEARCHES IN THIS BOOK

This book consists of thirteen research papers dealing with the isolation, identification and characterization of various chemical compounds from different medicinal plants, and/or with their pharmacological or clinical evaluation.

Lutomski has reported beneficial effect of *Allium sativum*, in a double-blind clinical trial, in human subjects suffering from hypertension and hyperlipidemia as it alleviated the psychic and physical symptoms characterstic of arteriosclerosis such as headache, giddiness, sleeplessness and flatulence, and lowered the blood cholesterol levels and the mild increase in the blood pressure.

Voelter has detected and structurally elucidated different flavanoids in different plants, namely - *Betulae folium, Crataegi flos* and *Folium, Ginkgo bilobal, Arnicae flos, Tiliae flos, Sophorae flos* and *Fagopyri herba*, and has described a new method for the quantitative spectroscopic detection of hydroxyethylated flavone glycosides in human blood and urine after intravenous and oral administration.

The hypoglycaemic activity of four active principles of *Trigonella foenumgraecum*, namely-trigonelline, orientin, vitexin and vetexin, has been investigated in mice by Hashim and associates. The drop in blood sugar by vetexin (0.5 mg/mouse) was similar to that of insulin. Although orientin (0.1 and 0.3 mg/mouse) did not produce a depth of reduction comparable to insulin, the duration of its effect was much prolonged. Trigonelline (0.2 mg/mouse) also reduced the blood sugar level and its effect persisted for 24 hours compared to 6 hours for insulin.

Gartaninxanthone from *Garcinia mangostana* has been tested for antiulcer and antimicrobial activities by Begum *et al.* The authors observed that xanthone markedly protected the gastric

ulcers induced by pyloric ligation in rats, and significantly reduced the gastric volume, and total and free acid. It also exhibited *in vitro* antibacterial effect against *S. aureus, S. typhi* and *E. coli,* and antifungal effect against *Microsporum canis.*

The root extract of *Smyrnium olusatrum* has been reported to exert promising antitumor activity both in *in vivo* and *in vitro* systems by Ulubelen *et al.* From this extract, two new sesquiterpene lactones of eremophilane type, have been isolated and named as istanbulin A and B. The authors have reported the oxidation mechanism of potential antitumor furanosesquiterpenes from several plants belonging to *Smyrnium* species.

The biological activity of some saponosides isolated mainly from *Aralia mandshurica, Calendulla officinalis* and *Beta vulgaris* has been investigated by Lutomski. All of them, oleanic acid derivatives from *Aralia mandshurica* and the same group of saponins from *Calendula officinalis* and those from *Beta vulgaris* were reported to decrease the total lipid level, the triglyceride level and the cholesterol level in serum and homogenised liver in Wistar rats. Further studies revealed that the oleanoside fractions had distinct antistress activity and exerted inhibitory influence on the animals motor activity and hexobabital sleeping period, and therefore, on the CNS. The influence of oleanosides was also studied on some immunological aspects, it was observed that while saposonides from *Aralia mandshurica* had fascinating effect, those from the remaining two plants did not stimulate the cellular response. Detailed investigations of oleanosides from *Aralia mandshurica* revealed that these compounds have distinct influence on immunological mechanism and may in futrue become the source of immunoregulating medicament.

The effect of bauerenol, a triterpene alcohol, isolated from *Eretia microphylla* has been investigated in immunopathalogical and inflammatory reactions by Nazimuddin and associates. The

authors observed that bauerenol suppressed the acute and chronic inflammation as evidenced by its ability to inhibit the carrageenin-induced hind paw oedema and cotton pellet-induced granuloma in rats; it also inhibited the systemic anaphylaxis, Schultz-Dale reaction, immunocytoadherence and the primary and secondary phases of adjuvant-induced arthritis in experimental animals.

The antiinflammatory and CNS depressant activities of xanthones from *Catophyllum trapezifolium* have been studied by Nazimuddin *et al.* Three xanthones, namely - dihydroxyxanthone, xanthone C and xanthone E were isolated and purified. The xanthones have been found to produce significant antiinflammatory activity in normal as well as adrenalectomised rats by both intraperitoneal and oral routes, and to produce mild degree of CNS depression characterised by ptosis, sedation, sleep, ataxia, and decrease in muscle tone and spontaneous motor activity, besides causing potentiation of pentobarbitone and ether-induced hypnosis.

Oksuz and Ayyildiz have isolated seven biologically active compounds from *Centaurea coronopifolia* and determined the chemical structure of four of these compounds. All compounds were germacren type sesquiterpene lactones and have α-methylen γ-lactone function. The authors opined that since this group is essential for cytotoxic action, it may be responsible for the promising activity of *Centaurea coronopifolia* against 3PS test system. The cytotoxic effect also of the glycosides obtained from *Ecballium elatarium* has been evaluated on the S-phase and L-strain cells by Ulubelen and associates.

The clinical trial of Ajmaline, a tertiary iodine base, isolated from *Rauwolfia serpentina* has been conducted by Ilyas in human subjects suffering from cardiac arrhythmias. Intravenous administration of Ajmaline was found effective in the termination of ectopic tachycardia. The author concluded that it is an effective

antiarrhythmic agent and is particularly effective in arrhythmias associated with Wolff-Parkinson- White syndrome.

Isolation and structural studies on the constituents of several medicinal plants have been carried out by Rahman and associates. The plants investigated were - *Berberis aristana, Fagonia indica, Buxus papilosa, Catharanthus roseus, Betula utilis, Cucumis prophetarum, Rhazya stricta,* and *Loranthus grewinkii.*

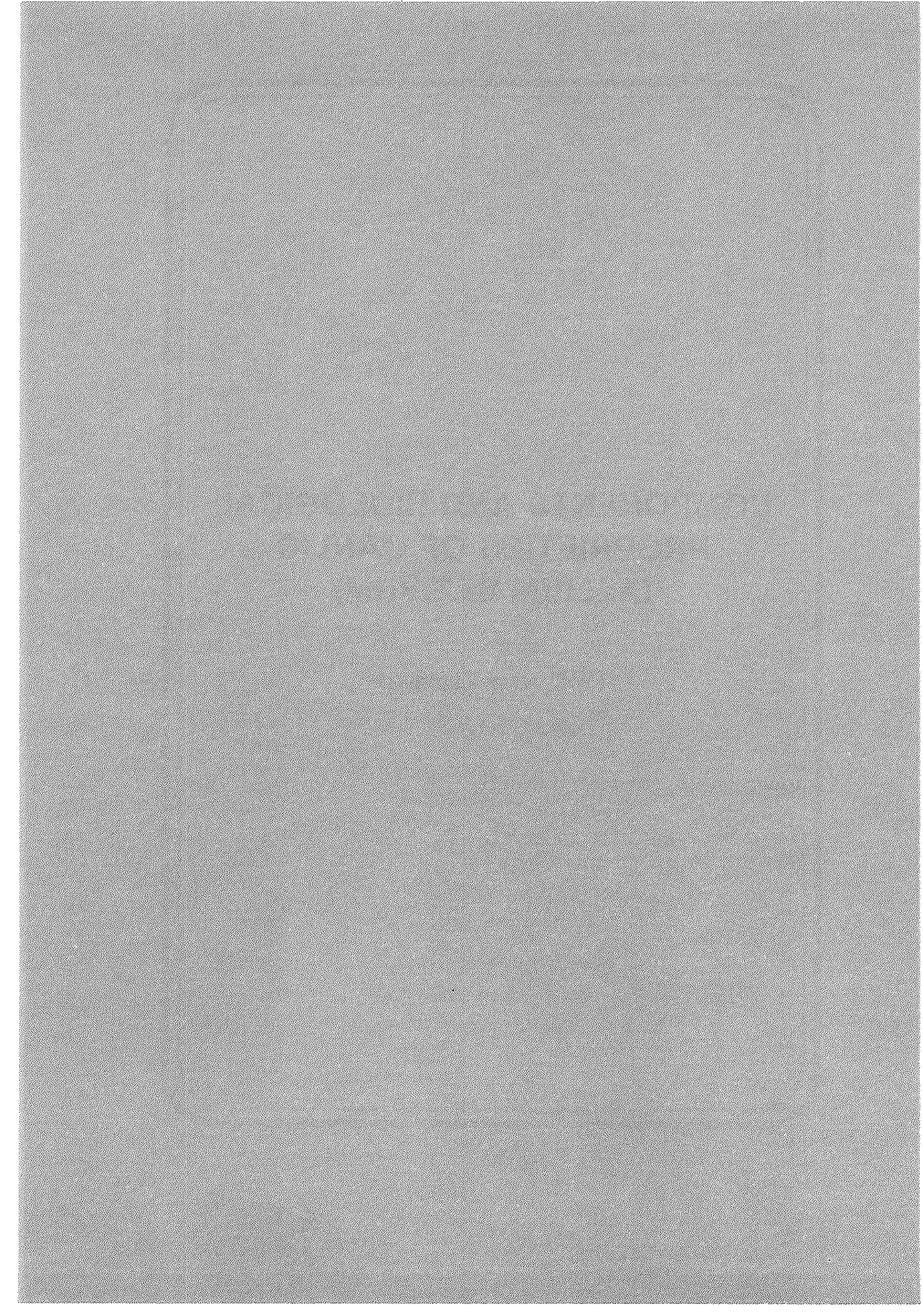
Phytochemical studies on new indole alkaloids have been conducted by Rahman and Rahman. *Rhazya stricta* contained Aspidospermidose, Bharhingine, Bisstrictidine, Didemethoxycarbonyltetrahydrosecamine, 17-Hydraoxyrhazisidine, 15 - β-hydroxyvincadifformine, Leepacine, Nb-methylstrictamine, Rhazigine, Rhazimine, Strictamine-N-Oxide, Strictanine, Strictanol, Strictícine, Strictimidine, Strictimine, Strictine, and Strictilamine; *Catharanthus roseus* contained Alioline, Bannucine, Fluorocarpamine-N-Oxide, Gomaline, Leurosinone, Rosamine and Rosicine; *Alstonia macrophylla* contained Alstonamide, Alstopicralamine, Alstozine-N-Oxide, Nb-Demethylalstophylline oxindole, 16-Hydroxy-Nb-demethylalstophylline, 19 - Hydroxyvincamajine, and Strictaminolamine; *Alstonia scholaris* contained Alstonamine, Scholaricine, and 19-20-Z -Vallesamine; *Ervatamia coronaria* contained Ervaticine, Hyderabadine, Lahoricine, Meharanine, and Stapfinine; and *Petchia ceylanica* contained Ceylanine, Desmethylpeceyline, (19R)-Epimisiline, and (19S)-Epimisiline.



COMPONENTS AND BIOLOGICAL PROPRIETIES OF GARLIC (ALLIUM SATIVUM)

Prof. Jerzy Lutomski

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COMPONENTS AND BIOLOGICAL PROPRIETIES OF GARLIC (ALLIUM SATIVUM).*

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From among several hundred species of the genus *Allium*, garlic plays the most important role in therapeutics. Grown by man for hundreds of years, garlic has been known not only because of its specific organoleptic properties but also because of its biological activity. The general therapeutic activity of garlic is ascribed to alkylpolysulfides - the derivatives of cysteine and to flavonoids, saponins, amino acids, vitamins and mineral salts. In literature, four main kinds of garlic activity are usually mentioned.

- antisclerotic [11,12,18,21,34]
- slightly lowering blood pressure [27,46]
- antibacterial, especially in infectious gastro-intestinal diseases[20,21,22]
- toning up old age disease[27,46] and lately probably also stimulating the psychophase/hypohyse/and the hormonal system[14].

COMPONENTS

Garlic includes volatile sulphuric compounds that, existing in the garlic oil, give it the characteristic unpleasant smell. Alliin - amino acid, the alkyl-derivative of cysteine that appears in quantities up to 0.2%[43], belongs to the main biologically active constituents of garlic[8,9,16,41].

Formulae of alliin[1] and allicin[2] are shown in Fig. 1.

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Alliin[1] is a non-active compound that only under the influence of the enzyme called alliinase transforms into allicin[2] - a pharmacologically active substance. Under the influence of the enzyme, the alkylsulfoxides of cysteine transform further into alkylpolysulfides and sulfides. Besides, the corresponding alkylsulfoxides of cysteine the final products of the enzymatic degradation are: pyroracemic acid and ammonia.

The enzymatic degradation of alkylcysteinesulfoxide are depicted in Fig. 2. In case when $R = R'$ some symmetrical derivatives arise, e.g. diallyl (allicin); when in turn $R = R'$ some mixed derivatives arise e.g. methylpropyl.

Another group of the organic sulphuric compounds characteristic of garlic consists of γ-glutamylpeptides, mainly derivatives of cysteine. The biological properties of those compounds have not been thoroughly explained yet. According to Virtanen[45], the compounds perform a certain role in the process of metabolism that stimulates the growth of plants. From among the compounds belonging to the group in question, Kominato[24] isolated a thioglycoside that he called scordinine. Scordinine is a complex of peptides consisting of fructuronic acid, allylmercaptan and a peptide that, besides other amino acids, includes also glutamic acid[29].

In the preventive treatment of arteriosclerosis one should apply medicines that hamper aggregations of thrombocytes. Substances of that kind exist also in garlic. They are: adenosine[25] and methylallyltrisulfide. Adenosine appears in many seasoning and medicinal plants. The concentration of adenosine in garlic is exceptionally high (56 mg%). Reuter, Deninger and Wagner[33] proved that adenosine hampers aggregation of thrombocytes and improves the flow of blood in coronary vessels.

In recent years, some other sulphuric components of garlic have also been proved to possess strong abilities to hamper the

aggregation. A group of Japanese research workers[2] have recently found a substance called methylallyltrisulfide that is responsible for the check of the aggregation.

The constituents mentioned above and other substances of garlic have been defined many times[1,19,25,26,31,34]. All of them have been presented in Table 1 A,B,C.

Once more I, would like to call your attention to the constituents that are important from the biological point of view. Those are: allicin(A) formed from alliin has strong antibiotic properties with the help of the enzyme alliinase; methyl-2-propenyltrisulfide that hampers the aggregation of thrombocytes (B), saponins with hypoglyceamic properties, flavonoids lowering blood pressure, scordinine and garlicin with antibiotic activity and adenosine that hampers the aggregation of thrombocytes (C).

ANTISCLEROTIC PROPERTIES OF GARLIC

There are still some more constituents of garlic, besides those mentioned above, that play an important antisclerotic role. Allicin and other sulfoxides influence the low density lipoprotein (LDL). Some constituents of garlic are generally considered to decrease the level of blood cholesterol and triglycerides.

Despite the fact that more and more experiments are done upon animals and people[10,12,] the question whether garlic may be administered as an efficatious remedy in the preventive treatment and therapy of arteriosclerosis, still remains unanswered. The problem was to be, at least partly, solved by the research supervised by the Polish Institute of Medicinal Plants and a clinical evaluation carried over by the West German garlic pharmaka called Ilja Rogoff ®/producer: Woelm-Pharma Eschwege, West Germany.

MATERIALS AND METHODS

The research was carried in ambulatory conditions upon 82 workers employed in one of the Polish Baltic-sea ports. The age of the patients ranged from 45 to 60. They were divided into two groups, group A and group B (Table 2). Patients from group A were treated with a drug of series A whereas patients from group B were treated with a drug of series B. It was done according to the principle of double blind trial, in congruence with the plan of randomization worked out in the Institute of Medicinal Plants in Poznan. The drug of series B was a placebo.

During each of the examinations

1. the clinical symptoms (headache, giddiness, sleeplessness, efficiency and general condition of the constitution, frame of mind, gastric disorders) were estimated by the patients themselves; a general estimation of the obtained results was done by the conducting doctor.
2. each of the examinations was accompanied with 3 psychological tests.
3. blood pressure and pulse were measured.
4. the following parameters were controlled: the levels of triglycerides, total cholesterol and sugar, fibrynolytic activity and electrocardiogram.

In the initial period of the research some of the patients showed moderate rise of blood pressure, especially the diastolic one, and heightened values of total cholesterol and triglycerides in the blood serum.

The drugs were administered 3 times a day, 2 pills before meal throughout the period of 12 weeks (1 pill included 50mg of Bulbus Alli. sat. sicc.).

Most of the examinations were done 4 times, thus: directly before the administration of the drug (initial research), after 4 weeks, after 8 weeks, and finally after 12 weeks.

The patients did not take any other medicines, neither in the course of treatment nor in the period preceding it (at least 2 weeks).

The results of the experiment, showing some differences within the course of treatment, were submitted to a statistic computer anlysis done by the so called Student's 't' Test.

RESULTS

The recapitulation of the observations made by the conducting doctor, proved the drug of series A to have a very beneficial influence upon subjective conditions of the patients. They have been relieved of headaches, giddiness, sleeplessness and gastric disorders. By comparison to the drug of series B, the drug of series A was found to have a better influence upon the subjective conditions of the patients. The drug turned out to be the most effective in case of headaches. As far as the improvement of subjective conditions was concerned the difference between the drugs of series A and B was the greatest (Fig. 3; Table 3).

A psychologic examination carried on with the help of three tests did not show any important differences within the compared groups.

The average frequency of pulse remained unchanged in the course of treatment in both groups (Table 4).

The average levels of blood pressure, both the systolic and diastolic (150/100 mmHg) in patients of group B did not show any features of statistic significance after 8 and 12 weeks of treatment. 17 patients of group A and only 8 patients of group B showed some normalization of blood pressure (Table 5).

The drug Ilja Rogoff ® with rutin lowered the systolic blood pressure on the average by 16mmHg, whereas the placebo drug (group B) lowered the systolic pressure only by 9.6 mmHg.

Contrary to group B, the average level of total cholesterol in group A was lowered significantly or on the limit of significance

(Tables 6 and 7). A comparison of average concentrations of lipids fractions in groups A and B led to the claim that the hypolipemic effect was similar in both groups.

The electrocardiographic examination did not contribute to the results of the experiment.

DISCUSSION

While comparing the results obtained after the administration of the Ilja Rogoff ® drugs of series A and B, one might notice a predominance of series A, especially within the range of the subjective symptoms registered by the conducting doctor. One observed the abatement of such symptoms as headaches and giddiness, flatulences and feeling of fulness in the abdominal cavity, and sleeplessness. Many patients asked for the repetition of the treatment with the drug of series A. That was, to a certain extent, a test prefering the pharmaceutical of series A.

As an exact comparative analysis[3] of the remaining results had shown, some hypotensional and hypolipemic effects were much more distinct with the patients of group A than with those of group B. The normalization of blood pressure, which was determined by some exactly established criteria, was observed after 12 weeks of treatment with the drug Ilja Rogoff ® in 77% of patients.

The fact that the level of cholesterol (Table 7) drops by 3.1% in group A and rises by 2.7% in group B, testifies the significance of differences[3] on the level of 0.5 and points to a beneficial drop of the level of cholesterol with the patients suffering from elementary hyperlipemia, as a result of the administration of the garlic pharmaceutical[4,5,6,12,18,31,42].

The observations of the influence of the garlic drug on the level of triglycerides did not give satisfactory results. In the course of numerous examinations[4,5,6,18,42] a distinct drop in the level of cholesterol and triglycerides was observed. Patkov[31] showed that

garlic may hamper the development of some specially hard forms of hyperlipemia.

The studies carried by Sainani[36] proved that garlic lowers the level of triglycerides in blood. In the patients with a raised sugar content in blood the statistic differences between groups A and B concerning the effect upon hyperglycaemia were not significant.

CONCLUSION

The comparison of the results obtained from the clinical experiments with the garlic pharmaka to the placebo, made according to the principle of a double blind trial showed very good effects in decreasing the symptoms of arteriosclerosis.

1. In many patients an improvement of psychic and physical conditions was observed; symptoms characteristic for arterio-sclerosis such as headache, giddiness, sleeplessness and flat-ulences were abated.
2. The tested drug lowered blood cholesterol, especially in patients with raised level of cholesterol.
3. The garlic drug was proved to possess also some hypotensional properties. After 12 weeks of its application normalization of blood pressure occurred with patients suffering from light hypertension.
4. Considering the vegetable origin of the drug in question and its perfect tolerance (no symptoms of its undesired activity were observed) one should state that garlic may be useful and efficient in the therapy and preventive treatment of arteriosclerosis.

TABLE I

Part A

THE ACTIVE CONSTITUENTS AND COMPOUNDS IDENTIFIED IN GARLIC

No.	Constituents		%	References
1.	Water		64.0	37
2.	Ash		1.45	37
3.	Cellulose		0.8	37
4.	Lipids		0.06	37
5.	Carbohydrates			20
6.	Muscuses			20
7.	Volatile compounds containing organic sulphuric compounds:		0.10-0.36	40,41,43
	a. alliin - amino acid			
	b. allicin-obtained from alliin with the help of the enzyme allii-nase, antibiotic properties			
	c. sulfides (mono):	dimethylsulfide		30
		2-propenlymethylsulfide		30
		di-2-propenylsulfide		30
	d. sulfides (di):	dimethyl disulfide		30
		methylpropyldisulfide		35
		methyl-2-propenyldisulfide		30,35
		dipropyldisulfide		35
		di-2-propenyldisulfide		20,35,44
		propyl-2-propenyldisulfide		36

TABLE I
Part B
THE ACTIVE CONSTITUENTS AND COMPOUNDS IDENTIFIED IN GARLIC

No.	Constituents		%	References
7.	e. sulfides (tri):	dimethyltrisulfide		30
		methylpropyltrisulfide		30
		methyl-2-propenyltrisulfide - hampers the aggregations of thrombocytes		2,30
		di-2-propenyltrisulfide		30
	f. thiols	methanethiol		30
	g. thiosulfonates	2-dipropenylthiosulfonate		19
	h. the remaining sulphuric compounds	sulphurdioxide		39
	i. alcohols	2-propen-1-ol		19
8.	Saponins	sitosterol glycoside with hypoglycaemic properties	0.0159	23,38
9.	Flavonoids	lowering blood pressure		7,25,38
10.	Vitamins	A,B_1,B_2,C,PP		15
11.	Vestiginal elements	Mg. Fe, Zn, Mn, Cu, Mo, Co, B, J		15
12.	Scordinine	thioglycoside of an antibiotic activity		24
13.	Garlicin	antibiotic properties		28
14	Adenosine	nucleoside hampering the aggregations of thrombocytes	0.056	17,33,34

TABLE I
Part C
THE ACTIVE CONSTITUENTS AND COMPOUNDS IDENTIFIED IN GARLIC

No.	Constituents	%	References
15.	Compounds with some properties of sexual hormones		15,41
16.	Choline	7.0	37
17.	Sinistrine of the insulin type		13,20,32
18.	Enzymes: alliinase, arginase, myrosinase, peroxidase, tyrosinase, desoxiribonu-clease		13,15,16

TABLE 2
CHARACTERIZATION OF THE 2 GROUPS OF PATIENTS

Group	A	B
The number of patients	44	38
Men	20	20
Women	24	18
Age (average)	45-60 (52)	45-59 (53)
Weight (average)	82 kg	76.5 kg
Height (average)	165 cm	166 cm

TABLE 3

THE ESTIMATION OF THE SYMPTOMS DONE BY THE PATIENTS

Symptoms	Improvement		No changes		Worsening	
	A	B	A	B	A	B
Physical condition	24 54.5%	14 38.8%	20 45.5%	20 55.5%	0 0%	2 5.7
Efficiency	16 36.4%	7 18.9%	27 61.3%	30 81.1%	1 2.3%	0 0%
Frame of mind	16 36.4%	6 16.2%	27 61.3%	30 81.1%	1 2.3%	1 2.7%
Sleep	25 59.5%	16 44.4%	16 38.1%	18 50%	1 2.4%	2 5.6%
Headache	26 66.7%	16 48.4%	13 33.3%	17 51.6%	0 0%	0 0%
Giddiness	21 56.8%	12 38.7%	16 43.2%	19 61.3%	0 0%	0 0%
Digestion	21 52.5%	13 37.1%	19 47.5%	22 62.9%	0 0%	0 0%
Average	51.8%	34.6%	47.2%	63.4%	1%	2%

Legend: A: Ilja Rogoff ® garlic pills; B: Placebo

TABLE 4

AVERAGE FREQUENCY OF PULSE/ MINUTE

Examination	Group A	Group B
Initial	75	76
After 4 weeks	74	75
After 8 weeks	74	76
After 12 weeks	74	76

TABLE 5

THE CHANGES IN BLOOD PRESSURE IN 36 PATIENTS SUFFERING FROM MILD HYPERTENSION

Blood pressure	Group A	Group B
Normalization	17 = 77.3%	8 = 57%
No changes	4 = 18.2%	4 = 28.6%
Worsening	1 = 4.5%	2 = 14.4%

TABLE 6

THE LEVEL OF TOTAL CHOLESTEROL (mg/100ml) AND TRIGLYCERIDES (mmol/L) IN BLOOD SERUM

Examination		Group A		Group B	
		cholesterol	triglycerides	cholesterol	triglycerides
Initial		212	2.52	221	2.38
After 4 weeks		204	3.32	223	2.61
After 8 weeks		202	2.72	217	2.15
After 12 weeks		205	2.40	225	2.50
P	1/2	0.05	0.1	0.5	0.6
	1/3	0.05	0.5	0.5	0.5
	1/4	0.05	0.02	0.5	0.5

TABLE 7

RECORD OF PATIENTS WITH HEIGHTENED VALUES OF CHOLESTEROL (mg/100ml) AND TRIGLYCERIDES (mmol/L) IN THE INITIAL PERIOD

Examination		Group A		Group B	
		cholesterol (n=5)	triglycerides (n=11)	cholesterol (n=5)	triglycerides (n=11)
Initial		276	3.88	290	3.83
After 4 weeks		255	2.86	301	3.27
After 8 weeks		257	3.95	252	2.63
After 12 weeks		275	3.20	287	3.37
P	1/2	0.1	0.05	0.4	0.05
	1/3	0.1	0.5	0.05	0.05
	1/4	0.6	0.05	0.05	0.1

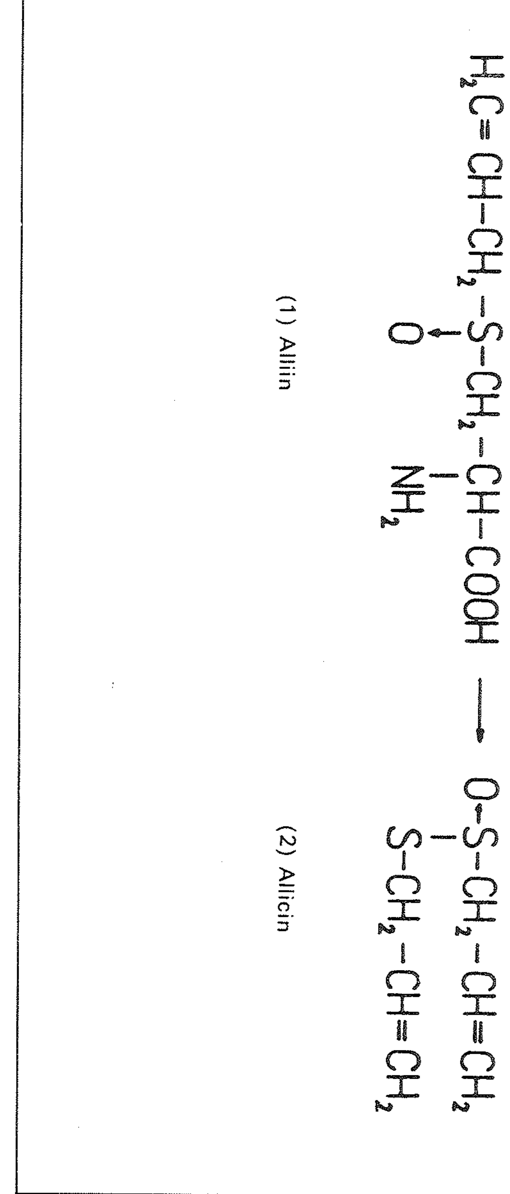


Fig. 1: Structural formulal of alliin (1) and allicin (2).

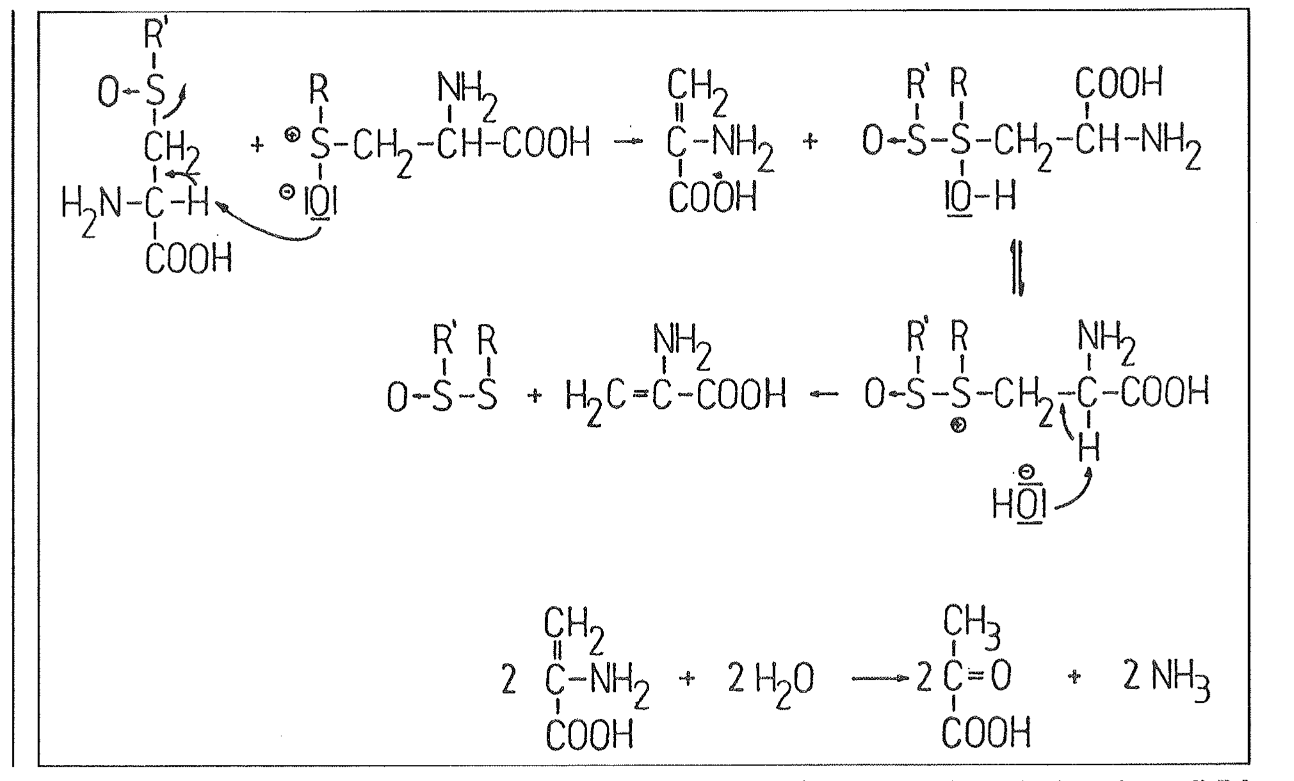


Fig. 2: The enzymatic degredation of alkylcysteinesufoxide. In case when R = R′ some symmetrical derivatives arise e.g. diallyl (allicin): when in turn R = R′ some mixed derivatives arise e.g. methylpropyl.

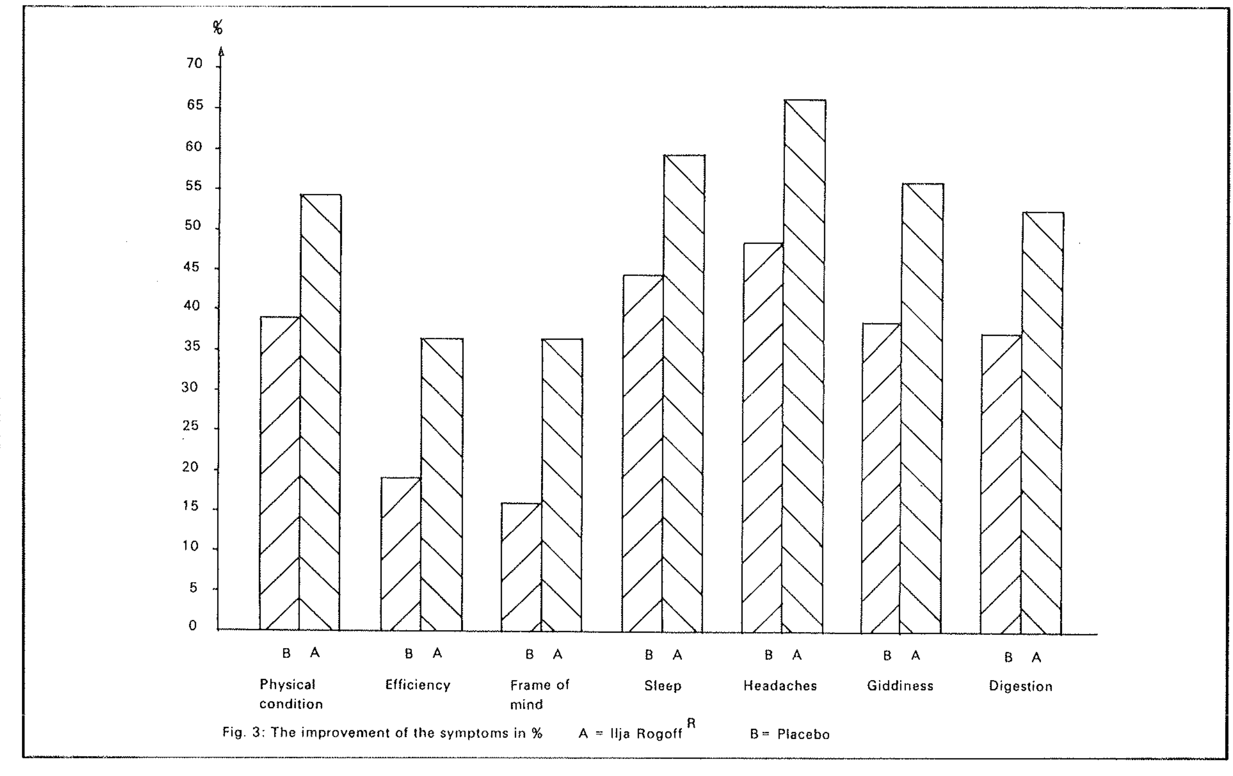


Fig. 3: The percentage improvement of the symptoms. A = Ilja Rogoff ® B = Placebo

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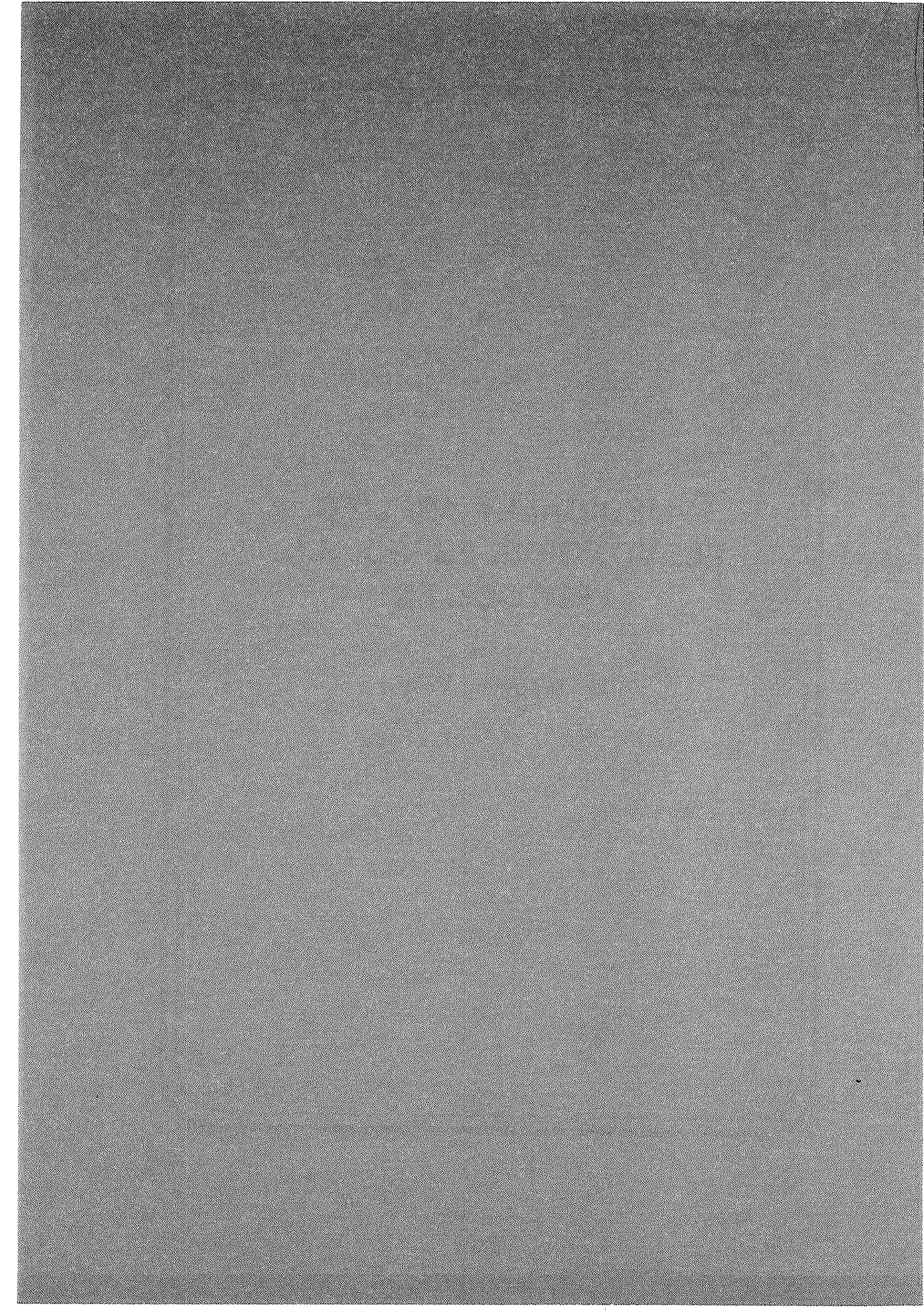
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MEDICALLY APPLIED FLAVONOIDS, ESPECIALLY RUTOSIDES

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INTRODUCTION

Already more than forty years ago Szent-Gyorgi and his co-workers[1] suggested that deficiency in flavonoids cause the disease scurvy. The substance, isolated from lemons or red peppers, influences the capillary permeability and was therefore designated as Vitamin P. However, it could be proven later that the compounds isolated by Szent-Gyorgi and co-workers[2] (citrin, a mixture of hesperidin and eriodictyol) have no vitamin character.

CHEMICAL STRUCTURES

The flavones (2-phenylbenzo-8-pyrone), isoflavones (3-phenyl-benzo-8-pyrone), flavonols (3-hydroxy-flavone), flavonones (2,3-dyhydroflavone) and flavonols (3-hydroxyflavone) are sub-groups of the naturally occurring flavonoids.

OCCURRENCE AND PHYSIOLOGICAL EFFECTS

The 8-pyrones occur in tissues of plants as glycosides and as free aglycones. Rich sources are leaves and blossoms, the concentration in roots, fruits and green woods is much lower.

The physiological importance of flavonoids is still a matter of discussion. Effects on enzyme activity, metabolism, liberation of

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histamine, capillary permeability, redox reactions and growth are discussed[3-11].

BIOSYNTHESIS

Investigations on ^{14}C -labelled compounds demonstrated that ring A of the benzopyrane is formed by three acetate units, ring B and three carbon atoms of the heterocyclic system have their origin from cinnamic acid, hydroxycinnamic acid (coumaric acid) and caffeic acid[12-14], respectivaly.

FLAVONOID DRUGS

Betulae folium: The leaves of the birch-tree have a relatively large content in hyperoside and myricetin galactoside. The drug is used for treatment of rheumatism and arthritis.

Crataegi flos and *Folium*: The dried blossoms and leaves of the all over Europe growing hawthorn have a two to three percent content of a whole series of flavonoids like quercetin, hyperoside, rutin, vitexin or rhammosyl vitexin. The drug is successfully applied for treatment of heart diseases and arterio-sclerosis.

Ginkgo bilobal: The leaves of the gingko-tree are rich in kaempherol, quercetin, luteolin and corresponding glycosides. Extracts are used to improve the blood supply.

Arnicae flos: The blossoms of *Arnica montana* L. contain several biologically active flavonoids like isoquercitrin, astragalin and luteolin-7-0 glucoside. Extracts are used for curing injuries and heart diseases.

Tiliae flos: Lime-blossom-tea is rich in quercitrin, isoquercitrin, astragalin. The tea is used for the treatment of colds and rheumatism.

Sophorae flos and *Fagopyri herba*: Both plants are used as raw materials for the isolation of rutin as they contain this flavonoid in a percentage of up to 25. Because of the oedema-preventing action

of rutin and especially its hydroxyethylated derivatives[15] large amounts of this flavonoid are needed.

METHODS FOR DETECTION AND STRUCTURE ELUCIDATION

In the past two decades, the methods for structure elucidation of natural products changed drastically by a series of different commercially available spectrometers. The parameters of proton nuclear magnetic resonance[16-21], carbon nuclear magnetic resonance[22-25], infrared spectroscopy[26], absorption spectroscopy[27], optical rotatory dispersion[28], circular dichroism[29-31], mass spectrometry and computer analysis[32-39] of a natural product nowadays often allow rapid unequivocal structure elucidation of an unknown compound.

As circular dichroism (CD) spectra can be measured from optically active compounds only, the method is mainly applied to natural organic compounds like terpenes, steroids, carbohydrates, amino acids, nucleosides etc. Since 1960, many stereochemical and conformational problems have been solved by means of the parameters of the CD spectra of these natural products.

Already a great deal of experience exists in the field of ^{1}H NMR and mass spectroscopy of flavonoids.[8,40] Most aglycones show intense molecular ion peaks. Interpretable mass spectra of flavone glycosides are received only from their trimethylsilyl, permethyl and trifluoroacetyl derivatives.[10,41]

With commercially available circular dichrosim apparatus for pyranose solutions no cotton effects can be measured even at wavelengths around 200 nm because the sugar chromophores absorb at 30-50 nm below 200 nm. However, if a sugar with different asymmetric carbon atoms is attached to an optically inactive chromophore absorbing at wavelengths > 200 nm an inherently symmetric, but asymmetrically perturbed, chromophore

is received. According to the theory of circular dichrosim cotton effects are expected in the wavelength range of the absorption band of a compound. Flavone glycosides usually show two major absorption bands in the region of 240 to 400 nm. Band I is expected between 280 and 380 nm and band II occurs in the spectroscopic range of 230 to 280 nm. The band located at higher wavelength is due to the absorption of the cinnamoyl system (ring B). The benzoyl system (ring A) causes absorption band II. As closely spaced cotton effects may have different signs and the rotational strength is related to the induced electric and magnetic dipole moments, often different electronic transitions of a chromophore are detectable by circular dichroism only and not by absorption spectroscopy.

According to our investigations the following conclusions can be drawn from CD spectra of flavone glycosides: 1) 3-0-glycosides show a characteristic strong positive cotton effect around 250 nm (band II range) if the bond is B-glycosidic. A neighbouring negative cotton effect is located around 230 nm and shows also strong intensity, 2) Flavone glycosides with sugars attached to ring B show much less characteristic patterns and 3) Flavone C-glycosides have characteristic dichronic properties as the asymmetric atoms of the carbohydrates are closer located to the flavone chromophore than in O-glycosides. Carbohydrates attached C-B-glycosidically to ring A show a characteristic strong negative cotton effect around 270 nm.

Valuable information is received from the comparison of the CD spectra of flavone glycosides measured in alcohol with those received in alcohol/$AlCl_3$ solution. To demonstrate these effects the absoption and CD spectra of myricitrin, 7, 4'-di-O-(B-hydroxyethyl) rutoside and 3', 4', 5, 7-tetra-O-(B-hydroxyethyl) rutoside are compared with each other. Flavones with hydroxyl groups at C-3 or C-5 or with an orthodihydroxyl system form complexes with aluminium chloride.[40] No complex formation with

aluminium chloride is therefore possible in the case of 3',4,4, 7-tetra-O-(B-hydroxyethyl) rutoside; the absorption and circular dichrosim spectra of this compound are therefore almost identical if recorded in ethanol or ethanol in the presence of $AlCl_3$. Flavones which contain hydroxyl groups at C-5 (e.g. 7,4'-di-O-(B-hydroxyethyl) rutoside, robinin, myricitrin) show in the presence of aluminium chloride a strong negative cotton effect around 280 nm followed by a strong positive CB-band around 258 nm. These data demonstrate clearly the utility of circular dichrosim to characterise flavone glycosides and to receive valuable information about the configuration and conformation of this class of natural products[42-45].

PHARMACOKINETICS OF HYDROXYETHYLRUTOSIDES

The pharmacokinetics of flavonoids in man are of fundamental interest with respect to their wide clinical application.[46-49] However, a clearcut and direct proof of the intestinal resorption of flavonoids remains still a difficult experimental task. There are several problems in the applicability and the detection limits of the classical chromatographic and spectroscopic methods. And the use of suitable labelled radioactive compounds depends on the success of complicated chemical synthesis and on the maximum doses allowed for clinical studies. Therefore, most of the *in vivo* experiments had to be done on animals. A recent paper on the metabolism of hydroxyethylrutosides reports compatible results on excretion after oral administration in man and in animals[50]. In these experiments ^{14}C-labelled hydroxyethyl groups were used.

In the following, a new method for the quantitative spectroscopic detection of hydroxyethylated flavone glycosides in human blood and urine after intravenous and oral administration is reported.

Hydroxyethylrutosides exhibit a pronounced negative cotton effect around 340 nm. This characteristic CD band corresponds to the wellknown UV absorption at that wavelength and its circular dichroic absorption can be taken as quantitative measure for the detection of hydroxyethylrutosides in solution. Human serum shows also a CD band at 340 nm. In contrast to the cotton effect of hydroxyethylrutosides this protein band is positive. Because of the opposite cotton effects of hydroxyethylrutosides and serum very small changes in the relative concentrations can be detected. Calibration curves revealed that one is able to detect amounts down to 0.1 mg hydroxyethylrutoside per 100 ml serum by direct measurements. There is no need for a chromatographic procedure, extraction, isolation or enrichment before the CD measurements. In order to make sure that there are no disturbing time-dependent phenomena like degradation the stability of hydroxyethylrutosides in serum was tested and no change of the wavelength or intensity of the 340 nm band was found up to 48h at 4°C. The CD will register only the intact flavone glycoside e.g. as soon as the sugar part is lost chirality is lost and the chromophore in the aglycone is no more detectable by CD. Therefore, this detection method is not only sensitive but also very specific. Simple numeric addition of the intensities of the CD band of pure hydroxyethylrutoside and serum solution and comparison to experimental values of solutions of the same concentration revealed that association effects of rutosides with serum proteins will lead to intensified chiroptical properties.

After injection of 1500 mg rutosides to male volunteers CD spectra of the serum and urine were taken. A strong decrease in the CD band with time is observed. With decreasing level of rutosides in serum an increase of their concentration in urine is observed. Pure urine has usually no cotton effects, and only few minutes after injection the CD spectrum of the drug can be recorded directly from urine probes. From various measurements, it can be concluded that

after i.v. administration a relatively large quantity of HR will be excreted unchanged in urine within 1-2 hours. After oral administration of 4gm hydroxyethylrutosides, a maximum in the concentration level is found several hours after application, and the drug can be detected by circular dichrosim up to 24 hours in blood. It should be mentioned that in the CD measurements also protein bound rutosides will be found. The cotton effect typical for hydroxylethylrutosides was not detected in urine during experiments with oral administration. From CD measurements of rutoside solutions in stomach juice it appears that the drug remains stable for several hours in this medium.

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THE HYPOGLYCAEMIC ACTIVITY OF FOUR ACTIVE PRINCIPLES OF TRIGONELLA FOENUMGRAECUM (TRIGONELLINE, ORIENTIN, VITEXIN AND VETEXIN) IN MICE

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INTRODUCTION

Trigonella foenumgraecum L. is found mainly in the Mediterranean region and is extensively cultivated in Southern Europe, Northern Africa and India (British Pharmaceutical Codex, 1934).

Trigonella foenumgraecum L. has been widely used in folk medicine for the treatment of various forms of malnutrition (Huerre, 1928) and for the promotion of lactation in nursing mothers (El-Ridi and El-Shahat, 1944). Morcos and El-Baradie (1955) reported its use for gastro-intestinal disturbances, habitual constipation, debility and diabetes mellitus, while Krishnaswamy *et al* (1971) and Voros and Nagy (1972) reported its use as an anti-microbial agent. Chemical studies have revealed that *Trigonella foenumgraecum* L. contains trigonelline, orientin, vitexin and vetexin (Wallis, 1962).

These therapeutic applications stimulated us to study and compare the hypoglycaemic activity of trigonelline, orientin, vitexin and vetexin.

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MATERIALS AND METHODS

Trigonelline, orientin, vitexin and vetexin were procured in powder form from the Faculty of Pharmacy of Cairo University. Each substance was tested at two different dose rates, and the whole investigation was carried out according to the method of Krawczynski and Osinski (1967). The potency of the drugs were compared with reference to insulin. Insulin was made up as a 10% solution in distilled water (Insulin manufactured by Merck in 1ml vial containing 20 units insulin).

Two hundred and twenty-five mice (mixed male and female) were used at a weight of 20-30 grams. The mice were prepared by with-holding food for 24 hours prior to injection of test substances and insulin. All four active principles and insulin were administered by intraperitoneal injection.

Each substance at each dose was tested on 25 mice. Blood samples were taken from the retro-orbital sinus of each mouse with a micropipette. Samples were taken before injection, and at 15 minutes, 30 minutes, 1,2,4,6,12,24 and 48 hours following injection, or until the blood sugar level had returned to its pre-injection level.

RESULTS

The results of the effects of the test substances on blood sugar levels in mice are shown in Table 1. Results for the insulin group are shown in Table 2. A comparison between the two dose levels of each of the four test substances and insulin is shown in Figures 1 to 4.

The administration of insulin at a dose of 1.0mg per mouse produced a very significant decrease in blood sugar level (BSL) to 102 $\pm$ 4.899 mg/dl after 2 hours which returned to pre-test level after 6 hours. Trigonelline at a dose of 0.1mg per mouse produced a drop in BSL to 136 $\pm$ 9.274 mg/dl after 2 hours, while a dose of 0.2 mg/dl caused the BSL to decrease to 116 $\pm$ 9.274 mg/dl after 6 hours, at both dose levels the BSL returned to normal after 24 hours.

Orientin reduced the BSL, in the 0.1 mg/mouse and the 0.3 mg/mouse groups, to 120 ± 8.367 mg/dl and 112 ± 5.31 mg/dl after 4 hours respectively. The BSL returned to pre-test levels after 48 hours.

Following administration of vitexin at dose levels of 0.1mg and 0.2 mg/mouse, BSL of 130 ± 13.785 mg/dl and 122 ± 9.696 mg/dl were recorded respectively after 2 hours, with a return to pre-injection levels after 6 hours. However, vetexin at dose levels of 0.2 and 0.5 mg/mouse produced a decrease in BSL to 121 ± 8.124 mg/dl and 106 ± 8.124 mg/dl respectively after 2 hours, with a return to normal levels after 6 hours.

All the above quoted blood sugar levels are significant statistically, at $p < 0.0002$.

DISCUSSION

In Egypt, fenugreek seeds are used as a popular household remedy for a variety of ailments (Morcos and El-Baradie, 1959)., Wallis (1962) reported that the seeds are used in veterinary medicine and occasionally as a spice in curry powders, and as an aqueous decoction are often used as a hot drink in cases of common cold (Dewidar, 1967).

T. foenumgraecum L. has received special interest in the field of pharmaceutical research. However, the literature lacks information relating to the properties of trigonelline, orientin, vitexin and vetexin.

The need to discover some inexpensive and harmless drug with anti-diabetic potency prompted investigation of these active principles of *Trigonella foenumgraecum* L.

The blood sugar levels of mice were determined before and after administration of the substances. It was found that all the four substances caused lowering of blood sugar levels to the following: Trigonelline at doses of 0.1mg and 0.2mg produced levels of

136 $\pm$ 9.274 mg/dl after 2 hours and 116 $\pm$ 9.274 mg/dl after 6 hours respectively. Vitexin produced blood sugar levels, after dosing at 0.1mg and 0.2mg, of 130 + 13.785 mg/dl and 122 $\pm$ 9.696 mg/dl after 2 hours respectively; Vetexin dosed at 0.2 and 0.5mg produced 121 $\pm$ 8.124 and 106 $\pm$ 8.124 mg/dl after 2 hours respectively; Orientin at doses of 0.1mg and 0.3mg, produced blood sugar levels of 120 $\pm$ 8.367 and 112 $\pm$ 5.831 after 4 hours respectively.

These results may be due to the blocking of the adrenal gland by the drugs, or they may be due to stimulation of the islet cells to produce insulin. The results obtained are comparable to those obtained by Morcos and El-Baradie (1959).

Lowering of the blood sugar may be due to an increased uptake of sugar by tissues such as muscle and fat. There is evidence indicating a decreased hepatic output of glucose through the action of insulin (Madison *et al*, 1960). It has been shown by hepatic vein catheterization in humans that the injection of insulin causes decreased hepatic output of glucose (Bearn *et al*, 1952 and Madison *et al*, 1960).

Of the four active principles of *T. foenumgraecum* L. tested, vetexin at a dose of 0.5 mg/mouse produced a drop in blood sugar levels similar to that observed after injection of insulin. Although the effects on blood sugar levels by orientin at doses of 0.1mg and 0.3mg/mouse did not produce a depth of reduction comparable to insulin, the duration of effect was more prolonged, returning to pre-test levels after 48 hours. Trigonelline at 0.2 mg/mouse also produced a blood sugar reduction that persisted for 24 hours compared to 6 hours duration for insulin.

More research is required before any of these substances can be properly evaluated as an antidiabetic agent, although some of these results show promise.

TABLE 1

The effects of some active principles of *Trigonella foenumgraecum* L. on blood sugar levels (mg/dl) following intraperitoneal injection in mice.

ACTIVE PRINCI-PLE	DOSE in mg		BEFORE DOSING	TIME AFTER ADMINISTRATION								
				15 mins	30 mins	1 hour	2 hours	4 hours	6 hours	12 hours	24 hours	48 hours
VITEXIN	0.1	MEAN ± S.E.	171 ± 1.0	140 ± 7.071 P < 0.0002	133 ± 11.136 P < 0.0002	132 ± 2.0 P < 0.0002	130 ± 13.785 P < 0.0002	153 ± 13.379 P < 0.0002	169 ± 1.66			
	0.2	MEAN ± S.E.	171 ± 1.871	150 ± 7.071 P < 0.0002	140 ± 6.325 P < 0.0002	138 ± 6.634 P < 0.0002	122 ± 9.696 P < 0.0002	129 ± 1.0 P < 0.0002	165 ± 2.236			
TRIGO-NELLINE	0.1	MEAN ± S.E.	172 ± 1.225	138 ± 8.603 P < 0.0002	137 ± 6.634 P < 0.0002	136 ± 2.45 P < 0.0002	136 ± 9.274 P < 0.0002	150 ± 6.325 P < 0.0002	170 ± 3.162	172 ± 1.225	171 ± 1.0	
	0.2	MEAN ± S.E.	170 ± 2.739	144 ± 8.718 P < 0.001	149 ± 6.783 P < 0.001	133 ± 2.0 P < 0.0002	120 ± 6.325 P < 0.0002	132 ± 6.634 P < 0.0002	116 ± 9.274 P < 0.0002	156 ± 5.099 P < 0.001	169 ± 2.45	
ORIEN-TIN	0.1	MEAN ± S.E.	171 ± 1.0	146 ± 7.484 P < 0.0002	140 ± 7.071 P < 0.0002	156 ± 7.484 P < 0.0002	126 ± 10.296 P < 0.0002	120 ± 8.367 P < 0.0002	152 ± 8.0 P < 0.0002	152 ± 8.0 P < 0.0002	164 ± 4.0 P < 0.01	170 ± 2.739
	0.3	MEAN ± S.E.	171 ± 1.0	142 ± 5.83 P < 0.0002	140 ± 6.325 P < 0.0002	116 ± 6.783 P < 0.0002	122 ± 8.603 P < 0.0002	122 ± 5.831 P < 0.0002	134 ± 2.45 P < 0.0002	134 ± 2.45 P < 0.0002	136 ± 10.296 P < 0.0002	169 ± 2.45
VETEXIN	0.2	MEAN ± S.E.	171 ± 1.0	153 ± 8.0 P < 0.0002	150 ± 9.354 P < 0.0002	134 ± 10.296 P < 0.0002	121 ± 8.124 P < 0.0002	153 ± 15.938 P < 0.0002	169 ± 1.0			
	0.5	MEAN ± S.E.	165 ± 2.236	144 ± 5.099 P < 0.001	124 ± 12.083 P < 0.0002	114 ± 9.274 P < 0.0002	106 ± 8.124 P < 0.0002	146 ± 8.124 P < 0.001	169 ± 1.0			

TABLE 2

The effects of insulin on blood sugar levels (mg/dl) following intraperitoneal injection in mice.

	DOSE IN mg		BEFORE DOSING	TIME AFTER ADMINISTRATION					
				15 mins	30 mins	1 hour	2 hours	4 hours	6 hours
INSULIN	1.0	MEAN ± S.E.	171 ± 0.979	158 ± 5.831 $P < 0.0002$	140 ± 4.472 $P < 0.0002$	112 ± 7.349 $P < 0.0002$	102 ± 4.899 $P < 0.0002$	154 ± 8.124 $P < 0.0002$	169 ± 2.449

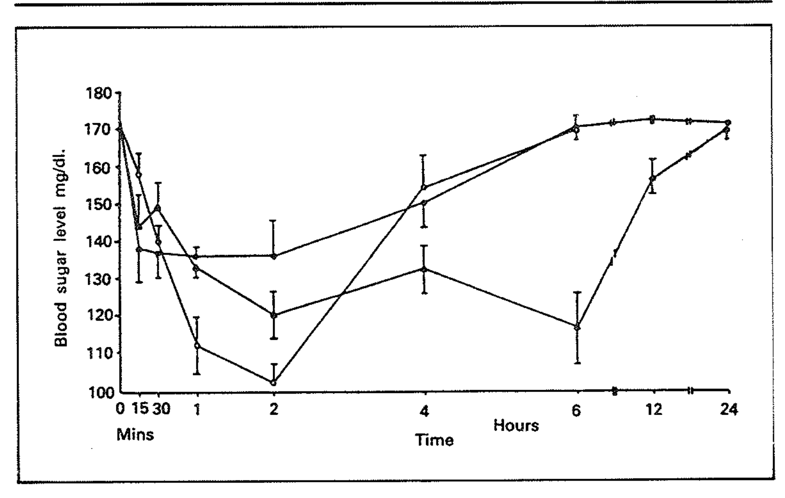


Figure 1: A comparison of the effects of trigonelline and insulin on mouse blood sugar. (●-●) trigonelline 0.1 mg; (▲-▲) trigonelline 0.2 mg; (o-o) insulin 1.0mg. Vertical bars represent ± s.e.m.

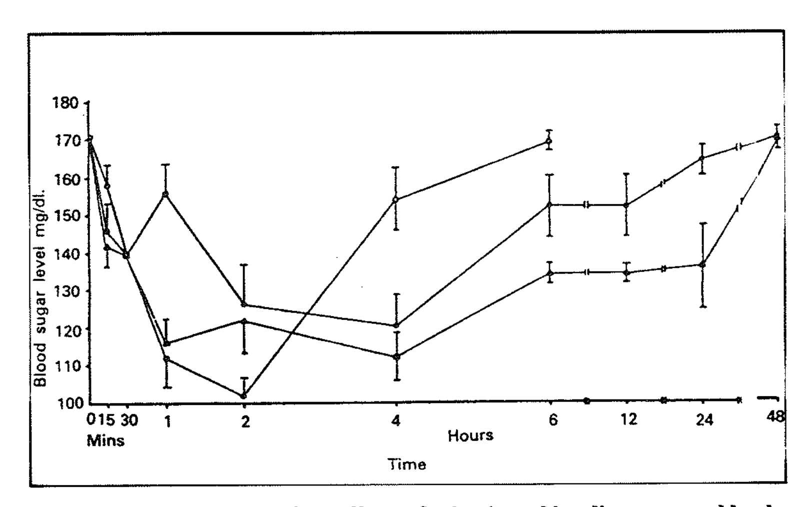


Figure 2: A comparison of the effects of orientin and insulin on mouse blood sugar. (●-●) orientin 0.1mg; (▲-▲) orientin 0.2mg; (o-o) insulin 1.0mg. Vertical bars represent ± s.e.m.

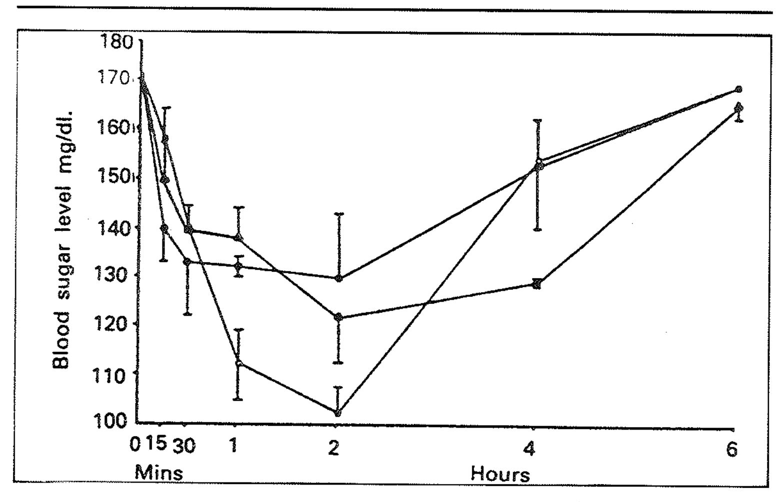


Figure 3: A comparison of the effects of vitexin and insulin on mouse blood sugar. (●-●) vitexin 0.1mg; (▲-▲) vitexin 0.2mg; (o-o) insulin 1.0 mg. Vertical bars represent ± s.e.m.

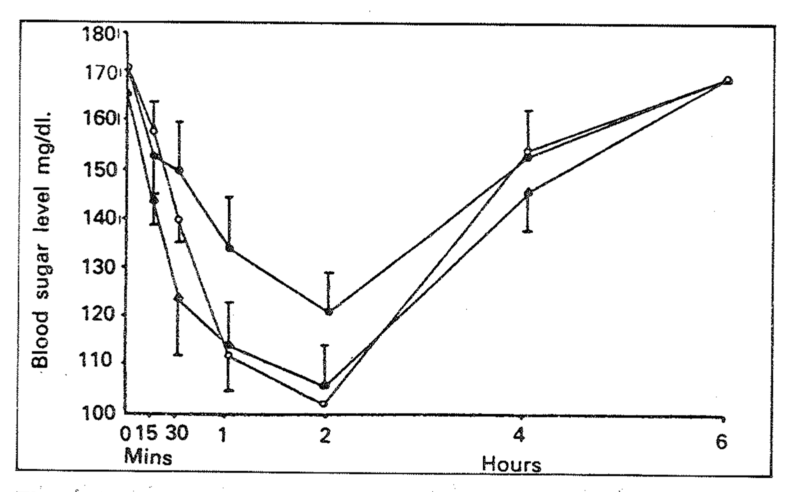


Figure 4: A comparison of the effects of vetexin and insulin on mouse blood sugar. (●-●) vetexin 0.2mg; (▲-▲) vetexin 0.5mg; (o-o) insulin 1.0mg. Vertical bars represent ± s.e.m.

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ANTI - ULCER AND ANTI MICROBIAL ACTIVITIES OF GARTANINXANTHONE FROM GARCINIA MANGOSTANA

Mrs. Nazeemunissa Begum, Dr. S.K. Nazimudin, Dr. C. Gopalakrishnan, Dr. D. Shankaranarayan and Dr. L. Kameswaran

INDIA

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INTRODUCTION

The tree *Garcinia mangostana* Linn. belongs to the family Guttiferae. The rinds of the fruit is an astringent and is useful in the treatment of diarrhoea and dysentery (Chopra).[1] In the recent days, new synthetic agents with specific pharmacological actions like histamine-2 (H2) receptor antagonists are widely used in the therapy of gastric ulcers, and after realisation of the importance of penicillin as a therapeutic medicine in about 1940, it gave a tremendous stimulus to the search of microorganisms capable of yielding new antibiotics and this search was also extended to cover the higher plants. In view of this and the reports on the anti-ulcer activity and antibiotic effects of certain xanthones of *Garcinia mangostana* (Shankaranarayan *et al*)[2], xanthones of *Calophyllum inophylum* and *Mesua ferra* (Gopalakrishnan *et al*)[3], anti-ulcer activity of xanthones of *Calophyllum trapezifolium* (Nazimuddin *et al*)[4] and antibiotic effect of a xanthone Morelline from *Garcinia morella* (Rao and Natarajan)[5], it was thought worthwhile to investigate the anti-ulcer, antibacterial and antifungal activities of gartanin - a xanthone from *Garcinia magnostana* Linn.

* Bulletin of Islamic Medicine, 2: 518-521, 1984.

MATERIALS AND METHODS

Isolation of xanthone gartanin: The isolation and purification of gartanin was done essentially according to the procedure of Kalayanaraman.[6] The rinds of the fruits of *Garcinia mangostana* was dried, powdered (10 kg) and extracted with hexane (5 x 10 lit). Solvent removal gave a yellow solid with some supernatant oil. The oil was decanted and the residue washed with small quantities of hexane. The remaining yellow powder was dried (12g). This showed two spots in TLC (Silica benzene: Methanol - 25:0.5). The faster moving spot resolved into a two coloured spot when the TLC was repeated in chloroform: hexane (90:10) and the plate exposed to iodine. The crude material (25g) was chromatographed over silica gel (1 kg) and eluted with chloroform: hexane (60:40). 25 ml fractions were collected. Fractions 10-18 were mixed and evaporated to give xanthone mangostin (200 mg). Fractions 19-29 gave a mixture of xanthone mangostin and gartanin (3 g). Fractions 30-41 gave pure xanthone gartanin (1 g). The mixture of xanthones mangostin and gartanin (3 gm) was rechromatographed over silica gel (250 g). Elution with chloroform: hexane (60:40) and collections in 10 ml fractions gave xanthone mangostin (300 mg) and xanthone gartanin (2.5g). The structure of gartanin is as follows:

O OH
OH
O
OH
GARTANIN

Drug preparation and administration

Since the xanthone gartanin was insoluble in water, fine suspensions of the above was prepared in 2% gum acacia using a Remi homogeniser at 3000 rpm.

Anti-ulcer activity

The preparation developed by Shay *et al.*[7] has been used for the present study. Two groups (six each) of Wistar albino rats were fasted for 48hr., and they were allowed to have only water *ad libitum*. One hour before the pyloric ligation, the group I animals were treated with distilled water which served as controls and to the group II animals was given gartanin (dose 50 mg/kg) intraperitoneally. The animals were then anaesthetised with ether and under aseptic conditions, a mild incision (1cm) was made below the xiphoid process and extended downwards. After cutting through the muscle layer, through the linea alba the stomach was exposed and the pylorus was ligated with a cotton thread. The cut ends of the muscle layer and skin were sutured. The animals were sacrificed after 18hr and the stomach was removed. The stomach contents were collected for examination and the stomach was opened along with the greater curvature, mounted on a cork board and the ulcers were examined and visually scored in arbitrary units of 0-4 according to severity (Bonny Castle).[8]

0 = normal, 1 = scattered haemorrhagic spots and hyperemia, 2 = deeper formed haemorrhagic spots and some ulcers, 3 = haemorrhagic spots and well formed ulcers and 4 = extensive haemorrhage, ulcers and perforation. Histopathological studies were also conducted by taking section of the stomach in both control and treated animals.

Measurement of volume of gastric secretion

The volume of the gastric contents was recorded. After the measurement of the gastric volume, the contents were centrifuged to get a clear supernatant fluid for determination of total and free acid.

Determination of free and total acids in gastric secretion

This was done by titrating an aliquot of the specimen filtrate with a standard solution of NaOH using 2 indicators in succession such as methyl orange and phenolpthalein.

In vitro antibacterial and antifungal effects

Test Organisms: A total of 8 bacteria belonging to various families and 4 species of fungi, both of yeast and filamentous type were used in the present study, such as the following:

BACTERIA: Staph. aureus, Pseudomonas aeroginosa, Proteus vulgaris, Klebscella pneumonia, E. coli, Vibro cholera.

FUNGI: Trichopyton mentagrophytes, Microsporum canis, Epidermophyton floccosum and *C. albicans.*

Methods of analysis

Three replicates were maintained for each of the tests carried out and each test was repeated to confirm the data obtained therein.

Cup-plate method: To find out the optimum concentration of the drug to be used, *S. aureus* and *T. mentagrophytes* were seeded in nutrient agar and Sabouraud's dextrose agar (SDA) respectively and poured in petri dishes. After solidification, wells were bored with cork-borer, each well was filled with different concentration of the drug prepared by serial dilution from an initial concentration of 1 mg/ml for bacteria and 10 mg/ml for fungi. The plates were incubated at 37°C for 48 hr. in case of bacteria and for one week at room temperature for fungi, and the results were recorded. A concentration of 100 ug/ml for bacteria and 5 mg/ml for fungi were found to be the optimal levels and these were used in further tests.

Superficial streak or point inoculation method: For evaluating the activity of the xanthone against bacteria and fungi, plates were poured with nutrient agar containing 100 ug concentration of the respective drug samples for bacteria and Sabouraud's dextrose agar 5 mg/ml slants containing the drug were prepared for testing fungi. After getting young cultures of bacteria tested earlier were streaked on the agar and incubated for 48 hr at 37°C. The results were recorded and compared. Activity on fungi was carried out in SDA slope cultures. To avoid overgrowth and cross contamination, one slant was used for each fungi tested. The agar slants were incubated at room temperature and the results were recorded after one week and compared.

Mic by tube dilution: Nutrient broth (2 ml) in test tubes were impregnated with the xanthone at the initial concentration of 100 ug/ml and was serially diluted up to 1.0875 ug/ml concentration. One loopful of the test bacterium from a young culture was added to each tube and shaken well. The tubes were incubated at 37°C for 48hr and the results were recorded. Uninoculated broth tube and the tube with no drug served as negative and positive controls respectively. The test was repeated thrice and the results compared and confirmed.

RESULTS AND DISCUSSION

Anti-ulcer activity

Microscopic examination of the incised stomach revealed the fourth degree of ulceration in the control rats characterised by extensive haemorrhage, ulceration and perforation. Animals treated with xanthone exhibited a marked protection against the ulcers induced by pyloric ligation. The ulcer scoring for the gum acacia treated rats was found to be 3.50 ± 0.27 while the animals treated with the xanthone gartanin was found to be 0.74 ± 0.24. The test animals exhibited only scattered areas of hyperemia and occasional haemorrhagic spots.

There was a significant difference in the total volume, total acid and free acid between the control and drug treated groups showing effective anti-secretory activity of the drug.

VOLUME		FREE ACID		TOTAL ACID	
Control	Test	Control	Test	Control	Test
11.46 ± 3.10	5.40 ± 0.73	5.66 ± 0.71	1.05 ± 0.74	11.86 ± 3.79	4.40 ± 1.21
$P < 0.001$ S		0.001 H.S.		0.01 S	

In vitro antibacterial and antifungal effects

The results on the effect of gartanin on the growth of bacteria *in vitro* showed that it had varying degrees of antibacterial activity, as tested by different assay techniques at an optimal concentration of 100 ug/ml. Gartanin was able to inhibit 3 out of 8 bacteria i.e. it inhibited *S. aureus, S. typhi* and *E. coli.*

As regards the antifungal activity the xanthone gartanin was active in inhibiting the growth of *M. canis* only.

The MIC for gartanin when tested for antibacterial activity was found to be 50-100 ug/ml.

ACKNOWLEDGEMENTS

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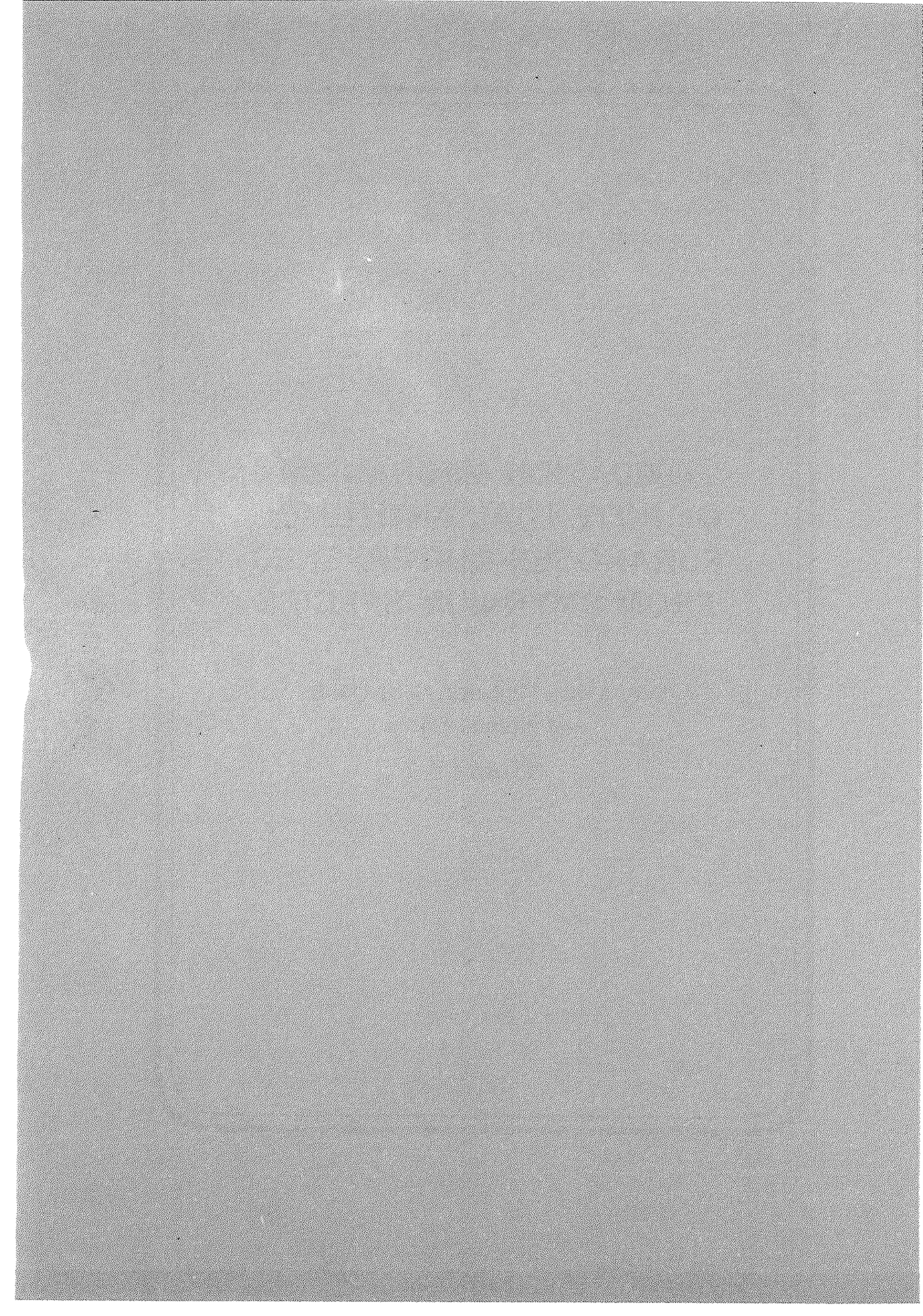
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OXIDATION MECHANISM OF POTENTIAL ANTITUMOR FURANOSES-QUITERPENES FROM SMYRNIUM SPECIES

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OXIDATION MECHANISM OF POTENTIAL ANTITUMOR FURANOSES-QUITERPENES FROM SMYRNIUM SPECIES*

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We have been screening Turkish plants for their antitumor activity for the last 15 years as a joint project with NIH (Washington D.C.). Until now about 200 plants were screened, some of them showed potential antitumor activity. Among them the root extracts of *Smyrnium olusatrum* showed a promising activity both in *in vivo* and *in vitro* systems. From this extract, two new sesquiterpene lactones of eremophilane type were isolated and they were named as istanbulin A (1) and B (2)[1].

1 R = OH istanbulin A

2 R = H istanbulin B

The roots of other *Smyrnium* species, namely *S. connatum*[2] and *S. creticum*[3] also yielded eremophilenolides, istanbulin C (3), D (4) and E (5) respectively.

* Bulletin of Islamic Medicine, 3: 458-461, 1984.

3 istanbulin C

4 istanbulin D

5 istanbulin E

In another study with the fruits of *S. olusatrum* a known compound glechomafuran (6) was obtained[4], this compound was found to be unstable especially in chloroform solution. The air oxidation of compound 6 starts in the first hour by dissolving in $CHCl_3$ and exposing to air and completes within 22 days. When the gummy residue fractioned on a Si-gel column, 3 lactones all germacrane type were obtained (7,8,9)[5].

Since none of the above compounds could be the precursor of eremophilenolides a systematic study with the roots and fruits of Smyrnium species present in Turkey was initiated, in order to prevent possible oxidation the plant materials were extracted with a mixture of light petrol and ether, evaporated under a vacuum at room temperature. The residues were separated in Si-gel columns

by fast elution. Using this technique, a furanoeremophilane (10) was obtained from the fruits of *S. cordifolium*[6], which probably is

the precursor of a number istanbulin type eremophilenolides. The same extract yielded furodiene (11), 2-acetylfurodiene (12) as well as oxidation products of furodiene, new eudesmanolides (13, 14)[7]. The biosynthetic oxidation mechanism is suggested as shown in Scheme 1.

11 R = H
12 R = OAc
13 R = H
14 R = OH

Scheme 1

On the other hand the roots of *S. cordifolium* yielded a different type oxidation poducts of furodiene, compounds (15, 16). These compounds have a new skeleton (Sheme 2) and named as smyrnicordiolides (15,16)[7].

Scheme 3

While the fruits of *S. rotundifolium*, in addition to several known sesquiterpenes yielded germacrane derivatives (17, 18, 19, 20) with a different oxidation path of furodiene (Scheme 3)[8].

Scheme 2

Compounds 13 and 14 were also isolated from the fruits of *S. olusatrum* and *S. qallaticum*[9,10]. The investigation is still going on and unstable compounds are being obtained, their structures will be discussed. The structures of all known and new compounds were established by spectral methods, for the new compounds spin-decoupling experiments as well as Dreiding models were used for stereochemical studies.

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BIOLOGICAL ACTIVITY OF SOME SAPONOSIDES

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BIOLOGICAL ACTIVITY OF SOME SAPONOSIDES*

Prof. Jerzy Lutomski
POLAND

Besides *Panax ginseng*, which was applied in oriental familiar medicine for centuries, *Aralia mandshurica* and *Eleutherococus senticosus* have gained some meaning in modern medicine recently. Although phamacological studies are constantly continued the adaptogenic, tonic, stimulant, anabolic and general strengthening effects of *Panax ginseng, Aralia mandshurica* and *Eleutherococus senticosus* are now well known. The most effective components of *Araliacae* species have been recently considered to be a saponin fraction. The compositions of these fractions differ much. *Panax ginseng* saponins consist mainly of 20-S-protopanaxadiol and 20-S-propanaxatriol glucosides. *Aralia mandshurica* saponins were found to be exclusively oleanolic acid derivatives. Meanwhile, we, in the Polish Institute of Medicinal Plants, in search of plant with approached biological properties to *Panax ginseng*, have limited our research entirely to some plants containing triterpenoid glucosides - exactly the derivatives of oleanolic acid. The triterpenoid glucosides are widespread in plants which belong to various families, and among the animals which belong to sea urchins/Echinodermata. The substances take place in vital organs and tissues. The triterpenoid glucosides demonstrate – same as exogenetic provenance material – a physiological activity in warmblooded animals. They influence metabolism, the state function of organs and of the whole organisms. That is why they are included into metabolism in biological systems and they demonstrate – as low molecular regulators – some polyfunctional properties.

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CHEMICAL CHARACTERISTICS OF TRITERPENOID GLUCOSIDES

The triterpenoid glucosides belong – according to the character of aglykon – to the following series: α – (fig. 1) or β – Amyrin (fig. 2), Lupon (fig. 3), Hopan (fig. 4), Dammaran (fig. 5), Lenostan (fig. 6), Holostantyp (fig. 7).

There can be the following monosaccharides in carbohydrate part: D-glucose, 3-O methyl- D-glucose, D-galactose, D-xylose, D-chinovose, L-arabinose, L-ribose, D-fucosa, α-rhamnose, Lyxose and D-glucuronic acid. They form one or two carbohydrate chains of linear or branched structure. Now, I will present some variants of classification of triterpenoid glucosides which consider several curiosities of carbohydrates.

After many investigations, one can accept that the appearance of triterpenoid glucosides in several plant families makes a chemitaxonomic stigma. The following families are especially rich in these substances: *Caryophylaceae, Compositae, Chenopoydiaceae* and others. The representatives of these last three mentioned families are:

Aralia mandshurica from *Araliaceae*,

Calendula officinalis from *(Compositae)*, *Astraceae* and

Beta vulgaris from *Chenopodiaceae*.

They have been examined in our Institute.

Aralia mandshurica: *Aralia mandshurica* is one of more important *Astraceae* plants. The saponosides from *Aralia mandshurica* possess aproximal inhibitory and stimulatory properties; as the root of *Panax ginseng*, for example. As the result of phytochemical investigations in the Institute of Medicinal Plants in Poznan, nine saponosides were isolated (with six unknown ones among them). All these compounds possess the same aglycone – oleanolic acid and therefore they were called oleanosides. There were five monosaccharides found in the carbohydrate part of the

substances mentioned above: L-arabinose, D-glucose, D-galactose and glucuronic acid.

Further procedural details of identification and isolation were presented in Polish Journal Herba Polonica in 1977.

***Calendula officinalis* L.:** Marigold (*Calendula officinalis* L.) from *Astraceae* was another saponin containing plant which we were interested in. The plant contains a rich fraction of oleanolic acid derivatives. The extract obtained from dried herb contained about 65% saponin compounds. Chromatographic analysis showed the presence of over 10 saponin compounds which aglycon was identified as oleanolic acid, after acid and alkaline hydrolysis. There were glucose, galactose and glucuronic acid found in the carbohydrate part.

We have also found some differences in the structure of carbohydrate parts of saponins from *Calendula officinalis* L. flowers and from its roots. There was glucuronic acid taking place at C-3 atom of oleanolic acid in flowers, whereas there was glucose in the same place of the compound in the plant's roots. Actually known saponosides are presented on fig. 8 for the flower extract and on fig. 9 for the root extract separately.

***Beta vulgaris* L.:** White beet (*Beta vulgaris* L.) from *Chenopodiaceae*, apart from a great number of organic nitric connections (i.e. 12 aminoacids), contains also betaine and pyrolidonocarboxylic acid. They are saponosides with oleanolic acid as aglycon. Three saponins have been isolated by Krecu (Soviet Union), and she defined them as saponosides A, B and C (fig. 10).

We also found a number of these compounds in the root of white beet, during our institute research. After their saccharose purification they were developed on column chromatograph with the use of polar extract agents. There were 11 compounds obtained, all of them having oleanolic acid as aglycon and glucuronic acid, glucose, arabinose and galactose in carbohydrate part, in different

quantity ratio. Apart from that, free oleanolic acid and its sodium were also found.

BIOLOGICAL CHARACTERISTICS

The isolated *Aralia mandshurica, Calendula officinalis* and *Beta vulgaris* fractions went under some pharmacological experiments carried out on animals. The goal of the experiments was to define the substance's influence on the lipid content in blood serum and homogenized liver in experimental hyperlipidemia in Wistar rats. Hyperlipidemia was evoked by a fatty diet. The studied substances were applied to stomach. All studied animals went under the following experiments:

a) Lipid determination in blood serum containing:
   (a) total lipids by the method of Postma and Stores,
   (b) triglycerides according to Eggstein and Kreutz,
   (c) total cholesterol by the method of Blaszczyszyn,
   (d) free fatty acids by the method of Duncombe,
   (e) β- lipoproteins after Koller and Bellaj;
b) Lipid determination in the homogenised liver (total lipids, cholesterol, triglycerides), by the same methods as for blood serum;
c) The determination of glucose level in blood serum by the o-toluidine method;
d) Liver weight determination.

The rats fatty diet containing coconut butter and cholesterol resulted in the increase of lipid level in blood serum and the homogenized liver. The increase was more distinct in liver, as far as total lipids, triglycerides and total cholesterol go.

All of them, oleanolic acid derivatives from *Aralia mandshurica* and the same group of saponins from Marigold herb and those from White beet (50mg/kg/ dose) were observed to decrease the total lipid level (21-37%), the triglyceride level (20-30%) and the

cholesterol level (17-25%) in blood serum and homogenised liver. The most active were saponin fractions from Aralia, then from Marigold and White beet.

Separate studies on the influence of oleanoside fractions on central nervous system depended on the measurement of rats spontaneous motility and their hexobarbital sleeping time period.

The experiments showed some distinct antistress activity, catecholamines regulation inducing role in brain structures, and inhibitory influence on the animals motor activity plus on the hexobarbital sleeping period, and therefore, on central nervous system.

Taking the mentioned pharmacological properties of the studied saponoside fractions into account, we may assume that, after some continued clinical experiments, oleanosides of Aralia and Marigold will especially be of some basis leading to their introduction to the market in the form of geronphitotherapeutics.

In recent years, the cause of senescen has been more often considered with the aspect of immunological reactions. Observations concerning aged people give much evidence for the immunological changeableness resulting in i.e. progressing infectional and cancer disease morbidity. Therefore, there was our goal to define the influence of the isolated oleanosides on some immunological aspects, in our further studies. We have observed that Marigold and the White beet saponosides do not stimulate cellular response.

Examinations of immunological properties of plant originating substances are conducted according to the III-stage scheme. In the first stage experiments possible influence of the substance on the humoral response is tested as well as on the cell mediated response, phagocytic system and regeneration abilities of cells after x-radiation.

The second stage experiments are the repetition of the positive change if parts already made, but they are considerably widened.

In the third stage there are conducted detailed directed experiments dealing with the influence of the plant originating substances on to the type of immunological response selected in the previous stages.

Oleanoside complex of *Aralia mandshurica* was tested on the basis of the above mentioned scheme with the reservation that the third stage has not been carried out up to now.

Oleanoside from *Aralia mandshurica* were obtained in the shape of yellow powder containing 40% of pure component. Experiments were made on mice, guinea pigs and rabbits administered this substance in water solution, orally, through the stomach wash in doses of 10 mg and 50mg per 1kg of weight.

Practically it was possible to define the toxic dose of oleanosides from *Aralia mandshurica* (OMA). The toxic influence either on to the organisms, or cells was not observed in the course of *in vivo* or *in vitro* experiments.

In cooperation with the scientists from the Immunological Department of the Medicine Academy in Szczecin, we made a series of immunological experiments:

1. Influence of OMA onto the dynamics of the phagocytosis process in animals. Examinations were conducted *in vitro* by means of isotopic method using as antigen sheep red blood cells (SRBC) labelled with chrome 51 as antigen, and by means of Wright method in the modification of Dolezal with the use of staphylococcal antigen.

Leucocytes and Schilling percentage formula were also counted. Six groups of rabbits were experimented on. They obtained OMA in doses of 10mg and 50mg per 1kg of weight and staphylococal vaccine in one dose of 1mg and repeated doses of 0.1ml. The most optimal results, it is the highest increase of ingestion and digestion of antigen by granulocytes were observed in the groups obtaining OMA repeatedly every day for 7 to 10 days in

doses of 50mg per 1kg of weight and also for 7 to 10 days in little doses of staphylococal vaccine. The further parameters of phagocytosis process we were interested in, were levels of leucocytes and particularly granulocytes. It was observed that application of OMA only for 10 following days in 50 mg per 1kg of rabbit weight caused statistically considerable decrease of the amount of leucocytes and particulary granulocytes 10 days after the completion of application of this substance. Such a decrease was not observed when OMA was applied for 10 following days simultaneously with staphylococal vaccine in little doses.

2. Next, influence of OMA onto the humoral response was evaluated by defining the number of cytoplasmatic cells in the spleen producing 19S antibodies by means of Jerne method in the modification of Sterzl and Mandel, and antibodies levels in blood: antistaphylococal, anti-*E. coli.* agglutinins and hemolysins by Adler method.

Examinations were made by applying OMA to mice and rabbits.

There was ascertained that OMA cause different organism humoral response depended on the animal species.

3. Testing of OMA influence onto the cell-mediated immune response was conducted by obtaining blastic transformation test using phytohemagglutinin, capillary migration test using phytohemagglutinin and tuberculin and skin test using 2,4,2 DNCB.

Influence of OMA onto the cell-mediated response within the range of blastic transformation process and production migration inhibitory factor were examined in 2 sets of tests namely by applying OMA *in vivo* or *in vitro*. It was found that OMA applied *in vivo* do not behave as mitogenic substances or do not stimulate production of lymphokine which inhibits migration of leucocytes. Those results were confirmed by examination of the delayed hypersensitivity reactions with the use of DNCB. There was no

observed increase of reactions in the groups obtaining OMA when compared with the control group.

4. There were made further evaluations of OMA influence onto the regeneration of spleen cells after x-irradiation of mice with sublethal doses of x-rays on total body. Examinations were made by giving OMA to mice before x-irradiation, or first mice were irradiated and next they obtained OMA. As far as regenerating spleen foci, spleen weight, spleen index and time of mice survival were considered it was observed that the best results were obtained by applying to mice OMA in doses of 50mg per 1kg of weight and irradiating them next.

5. There was examined the medium time survival of mice with implanted Ehrlich carcinoma, in the same experiment.The highest value was obtained in the animal group, administered 10 OMA doses before implantation of Ehrlich carcinoma.

6. Examination of OMA influence on the phagocytosis parameters in the animals given cyclophosphamide in one dose of 200mg per rabbit seemed to be an interesting experiment which proved protective influence of OMA on the phagocytosis process. These were observed the increased values of ingestion and digestion of antigen by granulocytes under the influence to OMA doses of 50mg per 1kg of weight for 10 following days. Protective influence of OMA on the phagocytosis process in rabbits obtaining hydrocortisone was not observed.

It may be possible that *Aralia mandshurica*, because of low toxity (210 mg/kg) and distinct influence on immunological mechanisms, will in future become the source of immunoregulating medicament.

However, further examinations are necessary.


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Fig. 8 FLOWER
A: R1 = glu/1-4/ gal/1-3/ gluos R2 = glu
B: R1 = glu/1-4/ gal//1-3/ gluos R2 = H
C: R1 = gal/1-3/gluos R2 = glu
D: R1 = gal/1-4/gluos R2 = H
E: R1 = glu/1-4/gluos R2 = H
F: R1 = gluos R2 = H

Figures 1-8.

Fig. 9 ROOT

R_1-O … $C=O$, $O-R_2$ (17, 28)

	R_1		R_2
1.	R_1 = gluc		R_2 = H
2.	R_1 = gal/1-4/gluc		R_2 = H
3.	R_1 = gal-gal/1-4/gluc		R_2 = H
4.	R_1 = gal/1-4/ + gluc/1-3/ > gluc		R_2 = H
5.	R_1 = gal/1-4/ + gluc-gluc/1-3/ > gluc		R_2 = H
6.	R_1 = gal-gal/1-4/ + gluc-gluc-/1-3/ > gluc		R_2 = H
7.	R_1 = gal/1-4/ + gluc-gluc-gluc/1-3/ > gluc		R_2 = H
8.	R_1 = gal/1-4/ + gluc-gluc/1-3/ > gluc		R_2 = gluc
9.	R_1 = 6'-O-methyl-glucs		R_2 = H
10.	R_1 = H		R_2 = Me

Fig. 10

R_1-O … $C=O$, $O-R_2$

A: R_1 = O-β-D-Gluc, R_2 = CH_3

B: R_1 = O-β-D-Gluc, R_2 = H

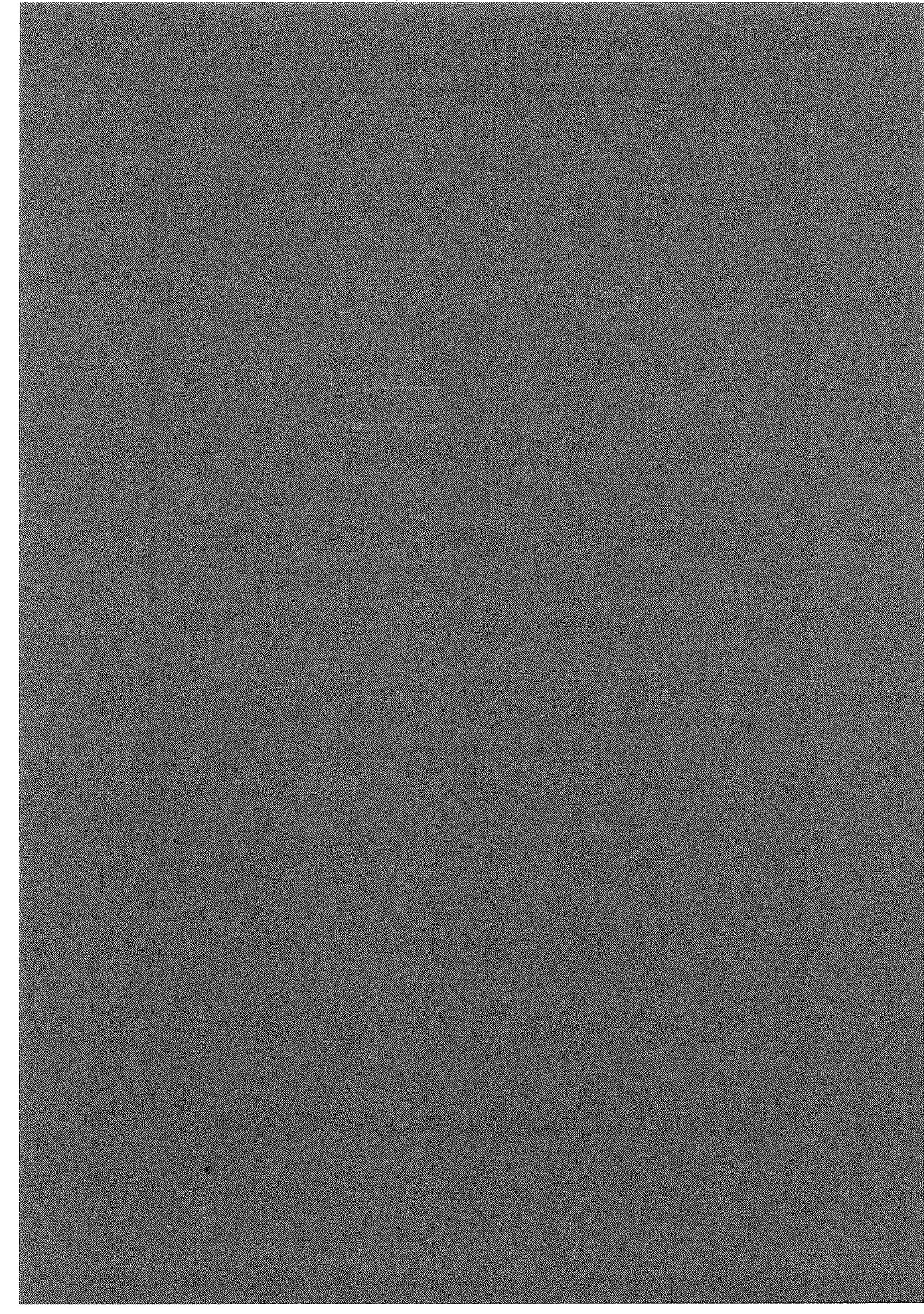
C: R_1 = Glucs, R_2 = H

Figures 9 and 10.

EFFECT OF BAUERENOL, A TRITERPENE ALCOHOL FROM EHRETIA MICROPHYLLA: IN IMMUNOPATHOLOGICAL AND INFLAMMATORY REACTIONS

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EFFECT OF BAUERENOL, A TRITERPENE ALCOHOL FROM EHRETIA MICROPHYLLA: IN IMMUNOPATHOLOGICAL AND INFLAMMATORY REACTIONS*

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INTRODUCTION

Ehretia microphylla Lam. (Family: *Boraginaceae*) is a medicinal small shrub which is used in syphilis, cachexia due to malignancy and as an antidote in vegetable poisoning. In Philippines, a decoction of the dried leaves is used for cough and stomach disorders and the leaves are used as a substitute for tea[1]. The plants belonging to family *Boraginaceae* are reputed to contain pyrrolizidine alkaloids which themselves are good anti-tumour agents. But, however, there appears to be no report on the isolation of pyrrolizidine alkaloids from this genus hetherto. Nazimuddin *et al*[2] have reported its anti-inflammatory activity. In view of this finding, it is of interest to elucidate the effect of bauerenol in various immunopathological and inflammatory reactions, since a number of non-steroidal anti-inflammatory agents have been reported to interfere with one or more discrete phases of immunopathological and inflammatory events[3].

ISOLATION AND IDENTIFICATION OF BAUERENOL

Air dried, powdered leaves with stem were extracted with hexane by cold percolation. Removal of the solvent and refrigera-

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tion left a crystalline solid in fair yields (0.1%). This solid gave positive Libermann-Burchard reaction. Chromatography over silica gel followed by repeated crystallisation from hexane gave bauerenol as colourless plates M.P. (206°-207°) (lit: 207°), the acetate melted at 292-94° (lit: 292-93°). All the compounds were characterised with IR and mass spectra. Oxidation of the alcohol gave a ketone, the mass spectrum of which was superimposable with the published spectrum of bauerenone[4]. Finally, it was confirmed by mixed melting point, mixed thin layer chromatography with an authentic sample of bauerenol. The first isolation of bauerenol was reported in 1958 from barks of *Acronychia baueni*[5].

HO

BAUERENOL

MATERIALS AND METHODS

Drug preparation and administration

Since triterpene monols are of lipid nature, they are soluable only in organic solvents such as benzene, chloroform etc., a fine suspension of the compound was prepared in 2% gum acacia using a "Remi Homogeniser" at 3000 rpm. The suspension was adminis-

tered at a volume of 2 ml/kg i.p. to guinea pigs and rats at a dose level of 50 mg/kg.

Animals

Inbred strains of Wister albino rats and guinea pigs were obtained from Madras Veterinary College , Madras.

Immunological studies

To evaluate the effect of bauerenol on humoral and cellular hypersensitivity reactions, the following parameters have been studied:

1. *Systemic anaphylaxis*: This was done according to the method of Harnath and Shyamalakumari[6] using guinea pigs of either sex (250-300 gm). The animals were sensitized with an i.p. injection of egg albumin in saline 4 g/kg. The animals were divided into 3 groups of 10 each and were administered with gum acacia, bauerenol (50 mg/kg) and dexamethasone (1 mg/kg) respectively, 2 days before the commencement of the experiment and subsequently for 6 days after the injection of the antigen. A challenging dose of egg albumin (4 g/kg) was administered (i.p.) after 10 days. Death of animals up to 24 hrs following the challenging dose was taken as lack of protection.
2. *Schultz-Dale's reaction* – Two groups of 5 guinea pigs each, were sensitized with egg albumin as described earlier. The control animals were administered with gum acacia and the test group with bauerenol (50 mg/kg), 2 days before the commencement of the experiment and subsequently continued for 6 days. Ten days later, all the animals were sacrified, and ileal pieces from both the test and the control groups were mounted in communicating organ bath containing Tyrode solution at 37°C. The tissues were exposed to the antigen-egg albumin and histamine at various concentrations, for a fixed time and the responses were recorded on a rotating smoked drum kymographically.

3. ***Immunocytoadherence*** or "***rosette formation***" – This method is based on the specific binding of an antigen with the surface of cells containing the corresponding antibody and the experimental procedures have been described by Sriram and Srinivasa Rao[7]. The heterogenous cells adhere strongly *in vitro* to the lymphoid cells containing the corresponding antibody on their surface and give rise to characteristic "rosettes", defined as any cell with more than half its circumference covered with red cells. Any decrease in the number of rosettes in comparison to control samples is taken as a positive response indicating possible interference in immune-reaction.

   Sensitization: Freshly collected sheep blood in Alsever's solution was centrifuged, processed according to the method of Sriram and Srinivasa Rao[7], the red blood cells were separated, standardised by counting in a haemo-cytometer, so as to give a concentration of 1×10^9 cells/ml and this was used for further studies. Three groups of albino rats (10 in each) were sensitized with sheep red blood cells (SRBC) at a dose level of 0.5 ml/ 100 gm,i.p., and the animals were administered with gum acacia, bauerenol (50 mg/kg) or dexamethasone (1 mg/kg), respectively for 7 days. The animals were sacrificed on the 8th day, the spleen was removed, washed free of clots and cut into small bits. The cells were separated by pressing the fragments with a glass rod in a test tube, washed with cold buffered saline and counted in a haemo-cytometer. The lymphoid cell suspension thus obtained was then treated with SRBC and the percentage of rosettes was counted using a haemo-cytometer and the results were interpreted according to the method mentioned above[7].

4. ***Adjuvant-induced arthritis*** – This was induced in 3 groups of albino rats (10 in each) by injecting killed mycobacterium tuberculosis cells suspended in paraffin oil (10 mg/ml), into the plantar region of the animals at a concentration of 0.1 ml/100 gm,

according to the method described by Edordo Arrigoni-Martelli[3]. The animals were administered with gum-acacia, bauerenol (50 mg/g) or dexamethasone (1 mg/kg), respectively 24 hr before the injection of the adjuvant and subsequently continued for 10 days. The primary reaction characterised by pronounced swelling of the injected paw, was observed within 4 hr after the injection of the adjuvant and persisted for several weeks. The oedema volume was measured plythysmographically on the 1st day, 5 hrs after the induction of oedema and subsequently on the 15th day. The paw volume of the contralateral limb was also measured on the 1st and 15th day. The delayed systemic response or the secondary response, was characterised by the swelling of the front paws, the contralateral paw and the appearence of arthritic nodules on the ear and tail. The ability of the test drug to inhibit the arthritic – syndrome was assessed and compared with the control and positive–control animals. The course of adjuvant–induced arthritis and the activity of the test compounds were also assessed by the grip strength method in which the ability of the animals to balance on a rotating rod (8 rpm) was tested as per the procedure of Bhide[8].

RESULTS

Systemic anaphylaxis

Pretreatment with bauerenol at 50 mg/kg for 2 days and subsequently for 6 days after sensitizing the animals, afforded 50 per cent protection against the mortality rate induced by the challenging dose of egg albumin on the tenth day after sensitization. While the gum acacia treated control animals died within 24 hours after the administration of the challenging dose of egg albumin, only 5 out of ten animals pretreated with bauerenol succumbed to the challenging dose, within 24 hrs. Only 3 out of 10 treated with

dexamethazone succumbed to the challenging dose of antigen, thereby showing 70% protection.

Schultz-Dale reaction

The isolated ileal tissue of guinea pigs pretreated with gum acacia responded by contracting powerfully when the antigen (egg albumin 1:100) was administered into the organ bath. The tissues also responded well to histamine (10 ng/ml). The ileal strips removed from the animals pretreated with bauerenol responded only very weakly to the administration of antigen, while the response to histamine was not altered to any significant extent.

Immunocytoadherance

While addition of sheep erythrocyte cell suspension to the lymphoid cell suspension of the nonsensitized animals did not produce rosettes, the addition of SRBC to the sensitized lymphoid suspension resulted in the formation of $45.5 \pm 9.4\%$ rosettes. Addition of SRBC to the sensitized lymphoid cell suspension obtained from animals treated with bauerenol suspension resulted in the formation of $45.5 \pm 9.4\%$ rosettes. Addition of SRBC to the sensitized lymphoid resulted in the formation of only $21.6 \pm 5.5\%$ rosettes. Similarly in the dexamethazone treated animals there was only $14.5 \pm 6.5\%$ rosettes ($p < 0.01$) thereby showing an inhibitory effect on immunocytoadherance.

Adjuvant-induced arthritis

Though there are a number of parameters for assessing the course of adjuvant arthritis such as i) arthritic lesions; ii) joint "score"; iii) hind paw size; iv) grip strength; v) paw temperature; vi) joint-histopathology; vii) erythrocyte sedimentation rate; viii) albumin/globulin ratio; ix) plasma fibrinogans; x) heat-coagulation protein (inflammation units) and xi) active lysosomal enzyme (Edoardo Arrigoni-Martelli, *loc. cit*), for the present study only the

hind paw size, as measured plethysmographically, and grip strength as tested by the ability of the animals to balance on the rotating rod (8 rpm) were estimated and comparative assessment was made between the animals treated with gum acacia and bauerenol respectively.

The results of the effects of bauerenol on adjuvant induced-arthritis in rats as evidenced by the measurement of paw size are given in Table 1. Bauerenol was highly active in inhibiting the primary as well as the secondary responses of the adjuvant-induced arthritis in rats. The additional parameter (grip strength method) to assess the course of adjuvant-induced arthritis and the activity of bauerenol in inhibiting the arthritic syndrome also confirms the activity of bauerenol. The results of the effect of bauerenol on adjuvant-induced arthritis as tested by grip-strength method are presented in Table 2.

DISCUSSION

Bauerenol, a triterpene alcohol is active in suppressing the "acute" as well as the "chronic" inflammations as evidenced by the ability of the triterpene alcohol to inhibit carrageenin-induced hind paw oedema and cotton pellet granuloma in albino rats[2]. Bauerenol also interferes with one or more discrete phases in the sequence of events leading to immunopathological and inflammatory responses as evidenced by the ability of the triterpene alcohol to inhibit systemic anaphylaxis, Schultz-Dale's reaction, immuno-cytoadherence and inhibition of the primary and secondary response in adjuvant-induced arthritis in experimental animals. Little evidence exists, in fact, that correlates clinical and experimental anti-inflammatory activity with immunosuppression. Among the several mechanisms of action proposed are i) interference with the synthesis of enzymes necessary for the release of inflammatory mediators; ii) interference with the pharmacological mediators of the inflamma-

tory responses; iii) reduction of complement levels and iv) inhibition of mononuclear cell exudation. Further studies are required to elucidate the activity of bauerenol with respect to the above in order to understand the precise mechanism by which bauerenol interferes with various inflammatory and immunological events. Anti-inflammatory, as distinct from immuno-supressive activity, has been described in several models of experimental inflammation for many immunosuppressive agents, such as 6-mercaptopurine, methotrexate, cyclophosphamide, chlorambucil and actinomycin-D. These drugs mainly act by preventing the participation of macrophages in delayed hypersensitivity and their infiltration into an inflammatory site. Similarly, a number of anti-inflammatory agents such as steroids, asprin like compounds, gold salts and pharmacological doses of oestrogen etc., have been shown to interfere with the immunopathological and inflammatory reaction such as adjuvant-induced arthritis. In view of the above findings, it would be interesting to extend such studies on bauerenol as an immunosuppressive agent and elucidate its role in various immunological reactions as well as in experimental tumours in animals models.

ACKNOWLEDGEMENT

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TABLE-1

Effect of bauerenol on adjuvant-induced arthritis in rats

(Values are Mean ± SD)

	Primary response. Injected paw volume (ml)					Secondary response. Contralateral paw volume (m1)		
	Initial	**5th hr**	**% Reduction**	**15th Day**	**% Reduction**	**Initial**	**15th day**	**% Reduction**
Control	1.26 ± 0.15	3.45 ± 0.22	–	2.45 ± 0.25	–	1.26 ± 0.15	2.05 ± 0.20	——
Bauerenol 50 mg/kg	13.50 ± 0.20	2.50 ± 0.25	27.5*	1.80 ± 0.25	26.53	1.35 ± 0.20	1.50 ± 0.10	26.8*
Dexamethazone 1 mg/kg	1.30* ± 0.10	2.20 ± 0.75	36.2*	1.60 ± 0.50	32.65	1.30 ± 0.10	1.40 ± 0.10	31.7*

* $P < 0.01$

TABLE -2

Effect of bauerenol on adjuvant-induced arthritis as tested by the grip strength method

(Method: Rota-rod (8 rpm); animal: Wistar rats. Values are Mean ± S.D.)

Group	Balancing in sec.
Control	130.5 ± 14.8
Sensitized control	52.8 ± 10.5
Sensitized rats treated with bauerenol	122.6 ± 18.6
Sensitized rats treated with dexamethazone	125.5 ± 16.4

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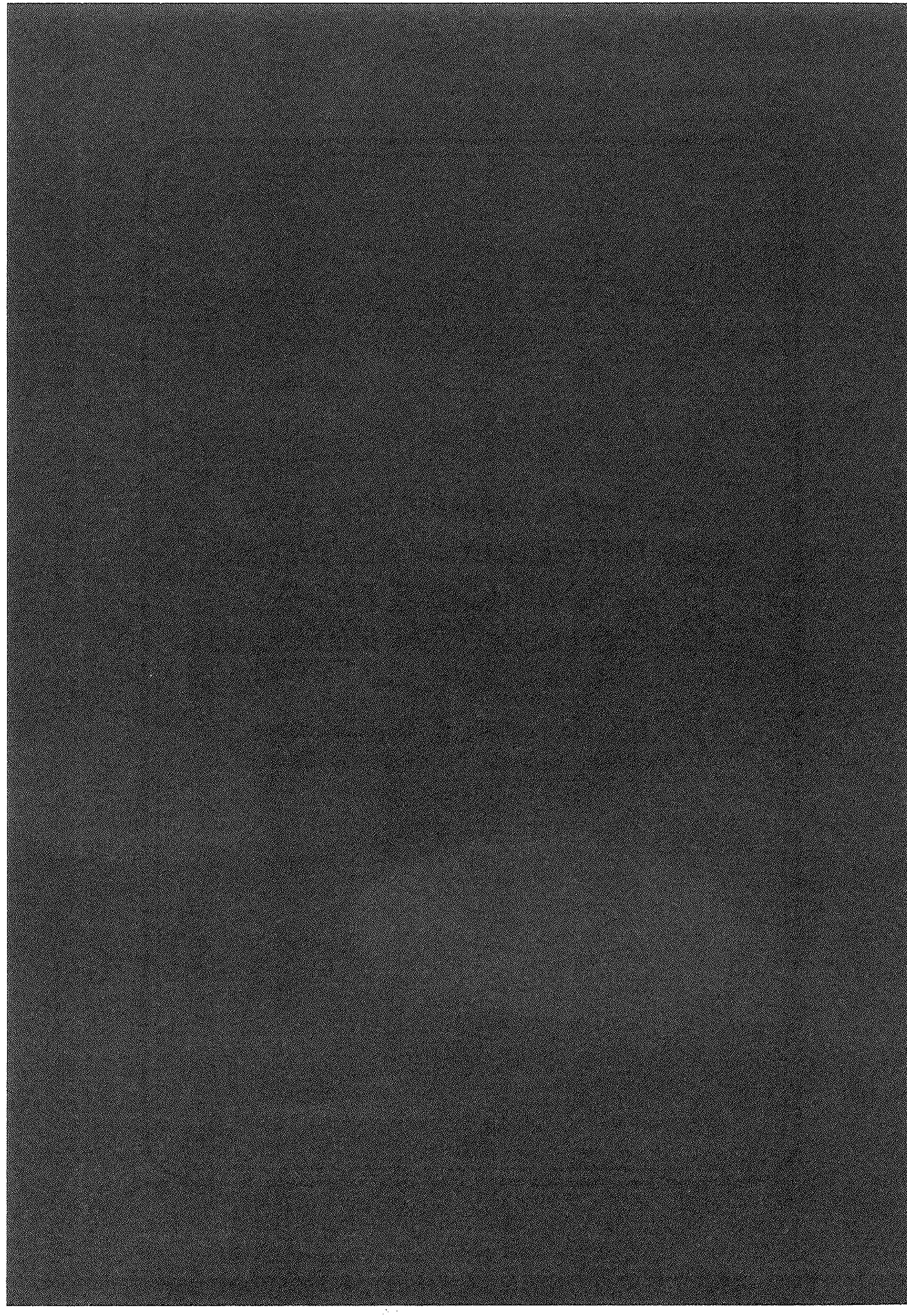
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ANTI-INFLAMMATORY AND CNS DEPRESSANT ACTIVITIES OF XANTHONES FROM CALOPHYLLUM TRAPEZIFOLIUM

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ANTI-INFLAMMATORY AND CNS DEPRESSANT ACTIVITIES OF XANTHONES FROM CALOPHYLLUM TRAPEZIFOLIUM*

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INTRODUCTION

The plant *Calophyllum trapezifolium* Th.W. belongs to the genus Calophyllum (Family *Guttiferae*). It is a moderate size evergreen ornamental tree. Various parts of these plants are commonly used in the treatment of rheumatism, skin diseases dysentery and bleeding piles etc. in the traditional systems of medicine (Nadkarni[1], Chopra, *et al*[2]). More than half a dozen Calophyllum species has been so far investigated. Kalyanaraman *et al*[3] have reported the occurrence of various xanthones from the heartwood of *Calophyllum trapezifolium* Th. W. In a collaborative study (Gopalakrishnan, *et al*)[4], the pharmacology of several xanthones of *C. inophyllum* such as jacareubin, desoxyjacreubin etc. was investigated and a variety of effects such as CNS depressant, anti-inflammatory, antimicrobial etc. have been reported. According to a review article of Hostemann and Wagner[5], not many investigations have been made on the pharmacology of xanthones and hence it was thought worthwhile investigating the pharmacology of the xanthones obtained from *C. trapezifolium*.

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MATERIALS AND METHODS

Isolation of the xanthones: Isolation and purification of the xanthones namely dihydroxy xanthones (DHX), xanthone C(XC) and xanthone E(XE) from the plant *C. Trapezifolium* were done essentially according to the procedures of Kalyanaraman[3]. The chemical structures of the xanthones and the abbreviation given in brackets given below, have been used whenever needed throughout this paper in describing the pharmacological action of the xanthones.

1-5 DIHYDROXY XANTHONE

XANTHONE E <X C>

XANTHONE. C <X E>

Drug preparation and administration: The xanthones of *C. trapezifolium* (DHX, XC and XE) were not freely soluble in water and hence fine suspensions of the compounds in 2% gum acacia (w/v) were prepared using a "Remi Homogenizer" at 3000 rpm. These were administered to rats and mice intraperitoneally and the volume of suspension was kept constant at 2ml/kg. The same volume of gum acacia suspension was administered to the control animals intraperitoneally.

Animals: Swiss albino mice (20-30gm) and Wistar albino rats (100-200gm) of either sex were used. They were fed with the standard pellet diet and housed for a week in the laboratory animal room.

Effects on Central Nervous System

a) *Gross Behaviour*: This was studied according to the method of Turner[6]. Xanthones of *C. trapezifolium* was administered to groups of 5 mice each in doses of 10, 30, 50, 100, 300, 800 mg/kg body weight. Gross behavioural changes were recorded at 15, 30, 60 and 120 min after the administration and were compared with the gum acacia treated control animals.

b) *Pentobarbital Sleeping Time:* This was studied in groups (10, in each) of albino rats. The test compounds were administered 30 min before the injection of pentobarbitone sodium (Nembutal, 30 mg/kg) intraperitoneally. The duration of sleep was assessed as the time between the loss and the return of the righting reflex. Results were expressed as per cent increase in sleeping time of the drug treated rats versus those of the control group.

c) *Ether Anaesthesia:* This was studied according to the method of Bhide[7] using pairs of rats, of which one animal was administered with gum acacia and served as control, while the other animal was treated with the test compounds at 100 mg/kg i.p. and exposed to 2ml of ether for 2 min in an inverted large funnel. The animals were removed immediately after 2 min, placed on the table and the time taken for regaining the righting reflex was recorded and the per cent potentiation of ether anaesthesia was calculated. For each group, 10 pairs were used.

d) *Forced Motor Activity:* This was studied according to the method of Dunham and Miya[8]. 20 Wistar rats of either sex were divided into 4 groups of which three belonged to test groups and the remaining one served as control.

Analgesic Activity

This was studied in groups (10 in each) of rats according to the method of Gujral and Khanna[2], using an analgesiometer. Tail flick response to radiant heat was observed in animals treated with gum acacia, xanthones (100 mg/kg) and morphine hydrochloride (15 mg/kg).

Anticonvulsant Activity: This was evaluated in rats as per the method of Dikshit, Tiwari and Dikshit[10] using a convulsiometer. Five groups (10 in each) of rats were subjected to maximal electroshock seizures (MES), by passing a current of 150V through a pair of corneal electrodes for 0.2 sec and the characteristic tonic extensor spasm of the hind limb was noted. Animals showing positive response only were used for the studies. The test, positive control and the negative control animals were administered with the xanthones (100 mg/kg), phenobarbitone sodium (Gardinal, 100 mg/kg) and gum acacia 30 min before the inducation of seizures respectively. The ability of the test compounds to abolish the tonic spasm of the hind limb was noted and compared.

Antipyretic Activity: This was studied essentially by the method of Maren[11] in rats. Pyrexia was produced by administration of Brever's Yeast (20% w/v, 1 ml/100 gm,s.c.). Peak temperature increase could be obtained only 18 hr after the administration of yeast. The ability of the xanthones (100 mg/kg) to decrease the elevated body temperature was compared with the control (gum acacia) and the positive control (paracetamol, 100 mg/kg) animals by using a clinical thermometer.

Effects on Cardiovascular System

Frog's heart in situ: This was studied as per the method of Burn[12]. The effect of graded doses of the xanthones on the rate and force of myocardial contraction was studied and recorded on a smoked drum.

Dog's blood pressure and myocardiogram: The effect of i.v. administration of graded doses of the xanthones on blood pressure and myocardiogram of anaesthetised dogs (5 animals) was recorded according to the method of Chushney[13].

Antiinflammatory Effect: The antiinflammatory effect of the xanthones was evaluated using albino rats by the following three different techniques:

1. Carrageenin induced hind paw oedema (Winter *et al*)[14].
2. Cotton pellet implantation (Winter *et al*)[15].
3. Granuloma pouch (Selye)[16].

For compounds which showed an antiinflammatory effect by parenteral administration, oral antiinflammatory effect was determined by techniques 2 only. In all the experiments the xanthones were administered at a dose level 50 mg/kg, while the positive control animals were administered with phenylbutazone (100 mg/kg) or dexamethasone (1 mg/kg). The control animals were administered with 2% gum acacia at a volume of 2 ml/kg. Antiinflammatory effect was expressed as per cent inhibition of the oedema volume/weight of granuloma tissue/volume of the exudate which was calculated by the formula (1-T/C) x100, where T and C are the mean value of the drug treated and control groups respectively.

Effect on adrenalectomised rats: Test compounds which exhibited an antiinflammatory effect in adrenal intact animals were studied for the effect in bilaterally adrenalectomised rats, in order to find out the involvement of adrenal glands in the mediation of the antiinflammatory effect. Bilateral adrenalectomy was done according to Zarrow, *et al*[17] and the antiinflammatory effect of the xanthone at a dose level of 50 mg/kg was examined according to the method of Winter, *et al*[15].

Effect on Peritoneal Mast Cells of Rat

Staining and counting of the ruptured and intact mast cells were done according to the technique described by Bray and Van Arsdel[18], in order to elucidate the effect of the xanthones on the rupture of mast cells induced by the mast cell degranulators such as compound 48/80, diazoxide and Triton-X-100.

Effect on prothrombin time

Two groups (10 in each) of albino rats were administered gum acacia, and xanthones (DHX, XC and XE) daily for 10 days. The plasma was collected from each animal on the 11th day and the prothrombin time was estimated according to the method of Quick[19].

RESULTS

Effects on central nervous system

a) *Gross Behaviour:* Preliminary screening of the xanthones of *C. trapezifolium* revealed that the xanthones have a definite modulating effect on the behavioural pattern of mice. All the xanthones were found to produce a mild degree of CNS depression characterised by ptosis, sedation, sleep, ataxia, decrease in muscle tone and decrease in spontaneous motor activity. The CNS depressant effect was predominent at a dose level of 200 mg/kg and this effect was observed within 15 min after the administration of the test compounds and the effect lasted for 60-90 min. All the xanthones up to a dose of 800 mg/kg did not produce any untoward symptoms and there was no mortality up to 24 hrs.

b) *Pentobarbitone sleeping time:* The xanthones exhibited varying degrees of potentiation of the pentobarbital sleeping time as evidenced by the data presented in Table 1.

c) *Ether anaesthesia:* Here again, the xanthones significantly potentiated ether anaesthesia as depicted in Table 2.

d) *Forced Motor Activity:* The effect of the xanthones on forced motor activity revealed that the compounds produce varying degrees of impairment, indicating loss of muscle tone (Table 3).

Analgesic, Anticonvulsant and Antipyretic Activities: The xanthones did not produce any of these activities in albino rats.

Effect on cardiovascular system: None of the xanthones employed in the present study had any effect on the rate and force of contraction of frog's heart as well as on the blood pressure and myocardiogram of dogs.

Antiinflammatory effect: The xanthones of *C. trapezifolium* produced significant anti-inflammatory effects in rats as tested by the carrageenin induced hind paw oedema, cotton pellet granuloma and granuloma pouch techniques. Table 4 summarises these results.

Antiinflammatory effect upon oral administration: Table 5 shows the antiinflammatory effects of the xarthones upon oral administration.

Antiinflammatory effect in adrenalectomised rats: Table 6 shows the results of the antiinflammatory effects of the xanthones in adrenalectomised rats.

Effect on rat peritoneal mast cells in vitro: The xanthones did not show any significant mast cell membrane stabilising effect, as evidenced by their inability to prevent the rupture of mast cell induced by polymyxin B, diazoxide and Triton X-100.

Effect on prothrombin time: While the prothrombin time in the control rats was 12 ± 0.62 sec, the prothrombin times of DHX, XC and XE were in the orders of 12.2 ± 0.66 sec, 12.0 ± 0.28 sec and 12.4 ± 0.48 sec respectively, which were not statistically different from the control values.

DISCUSSION

The xanthones of *C. trapezifolium* have been found to produce a variety of interesting pharmacological effects in experimental animals. The findings of the authors are in agreement with the findings related to *Garcinia mangostana, Calophyllum inophyllum and Mesua ferrae (*Shankarnarayan, *et al)*[20], (Gopalakrishnan, *et al)*[4]. While the xanthone -c- glucoside such as mangiferin and xanthone -o- glucoside such as mangostin, 3, 6, dio-glucoxide have been reported to produce CNS stimulant, analgesic, anticonculsant (Bhatacharya, *et al)*[21] and cardiotonic effect (Shankarnarayan, *et el)*[22]. No such activities could be observed for the xanthones of *C. inophyllum* and *M. ferrea* (Gopalakrishnan, *et al)*[4] and xanthones of *C. trapezifolium* studied at present.

The xanthones used in the present study have been found to produce significant antiinflammatory activity in normal as well as in adrenalectomised rats by both intraperitoneal and oral routes. The slight decrease in the antiinflammatory activity of all the xanthones upon oral administration suggests that there might be some impairment in the rate of absorption of the xanthones from the gastrointestinal tract which requires further study. The exact mechanism by which the xanthones produce antiinflammatory activity is not known. Unlike indomethacin and meclofenamate, xanthones of *C. trapezifolium* do not stabilise the mast cell membrane as evidenced by their lack of antagonism activity against various mast cell degranulators. Since the xanthones mangostin has been reported to inhibit prostaglandin synthetase (Shankaranarayan)[20], it would be interesting to elucidate the effect of the xanthones of *C. trapezifolium* on the activity of prostaglandin synthetase.

Though the antiinflammatory agents in clinical use exhibit analgesic and antipyretic properties, the xanthones used in the present study do not possess analgesic properties.

Quite interestingly, the xanthones used in the present study do not produce any prolongation of prothrombin time in rats, which is a common side effect encountered with the antiinflammatory compounds such as acetylsalicylic acid.

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TABLE I

EFFECT OF XANTHONES OF *C. TRAPEZIFOLIUM* ON PENTOBARBITONE SLEEPING TIME

Drug	Dose	PBS TIME MEAN ± S.D.	% Increase
Gum acacia	2 ml/kg	77.30 ± 6.60	-
DHX	100mg/kg	93.6 ± 6.3	19.8*
XC	100mg/kg	101.5 ± 8.5	31.3*
XE	100mg/kg	105.6 ± 9.2	36.3*

*P = < 0.01

TABLE II

EFFECT OF XANTHONES OF *C. TRAPEZIFOLIUM* ON ETHER ANAESTHESIA

Drug	Dose	Ether Anaesthesia Mean ± SEM	% Increase
Gum acacia	2 ml/kg	4.25 ± 1.25	-
DHX	100mg/kg	6.25 ± 1.28	47.05*
XC	100mg/kg	7.25 ± 1.48	70.50*
XE	100mg/kg	8.60 ± 1.65	102.60*

*P = < 0.01

TABLE III

EFFECT OF XANTHONES OF *C. TRAPEZIFOLIUM* ON FORCED MOTOR-ACTIVITY

Drug	Dose	Balancing time Mean ± SEM	% Decrease
Gum acacia	2 ml/kg	126.80 ± 4.41	-
DHX	100mg/kg	82.80 ± 6.55	34.7*
XC	100mg/kg	76.50 ± 5.60	39.7*
XE	100mg/kg	71.50 ± 6.20	43.6*

*P = 0.01

TABLE IV

ANTI INFLAMMATORY EFFECTS OF XANTHONES OF *C. TRAPEZIFOLIUM*

DRUG	HIND PAW OEDEMA VOLUME (ml)			COTTONPELLET GRANULOMA WT OF COTTON PELLET (mg)			GRANULOMA POUCH VOL. OF EXUDATE (ml)		
	MEAN ± SEM	% Reduction	P	Mean ± SEM	% Reduction	P	Mean ± SEM	% Reduction	P
Gum acacia	1.57 ± 0.17	-	-	55.3 ± 6.7	-	-	2.76 ± 0.54	-	-
Dexamethasone	0.49 ± 0.15	68.8	(0.001)	26.6 ± 3.8	52.3	< 0.001	0.90 ± 0.20	67.4	< 0.001
Phenylbutasone	0.46 ± 0.11	70.7	(0.001	25.3 ± 6.0	54.3	< 0.001	0.98 ± 0.34	64.5	< 0.001
DHX	0.98 ± 0.11	37.6	(0.01	37.5 ± 4.1	32.2	< 0.01	1.66 ± 0.52	39.9	< 0.01
XC	0.91 ± 0.18	42.0	(0.01	34.1 ± 5.2	38.3	< 0.01	1.49 ± 0.24	46.0	< 0.01
XE	0.83 ± 0.15	37.1	(0.001	29.5 ± 4.6	46.7	< 0.001	1.38 ± 0.25	50.0	< 0.001

TABLE V

ANTIINFLAMMATORY EFFECT OF THE XANTHONES OF *C. TRAPEZIFOLIUM* UPON ORAL ADMINISTRATION IN RATS

Drug	Dose mg/kg	Carrageenin induced hind paw oedema, vol. in ml, Mean ± S.D.	% Potentiation
Gum acacia	2 ml	1.62 ± 0.20	-
Phenylbutazone	100	0.54 ± 0.16	66.7*
DHX	50	1.22 ± 0.18	24.7*
XC	50	1.16 ± 0.22	28.4*
XE	50	1.11 ± 0.17	31.5*

*P (0.01)

TABLE VI

ANTIINFLAMMATORY EFFECT OF THE XANTHONES OF *C. TRAPEZIFOLIUM* IN ADRENALECTOMISED RATS

Drug	Dose mg/kg	Cotton Pellet Granuloma, wt. of cotton pellet in mg., Mean ± S.D.	% Potentiation
Gum acacia	2 ml	56.6 ± 6.4	-
Dexamethasone	1	28.7 ± 7.0	49.3
Phenylbutasone	100	32.0 ± 6.0	43.5
DHX	50	42.4 ± 5.8	24.9
XC	50	40.2 ± 6.2	29.0
XE	50	34.5 ± 5.6	39.1

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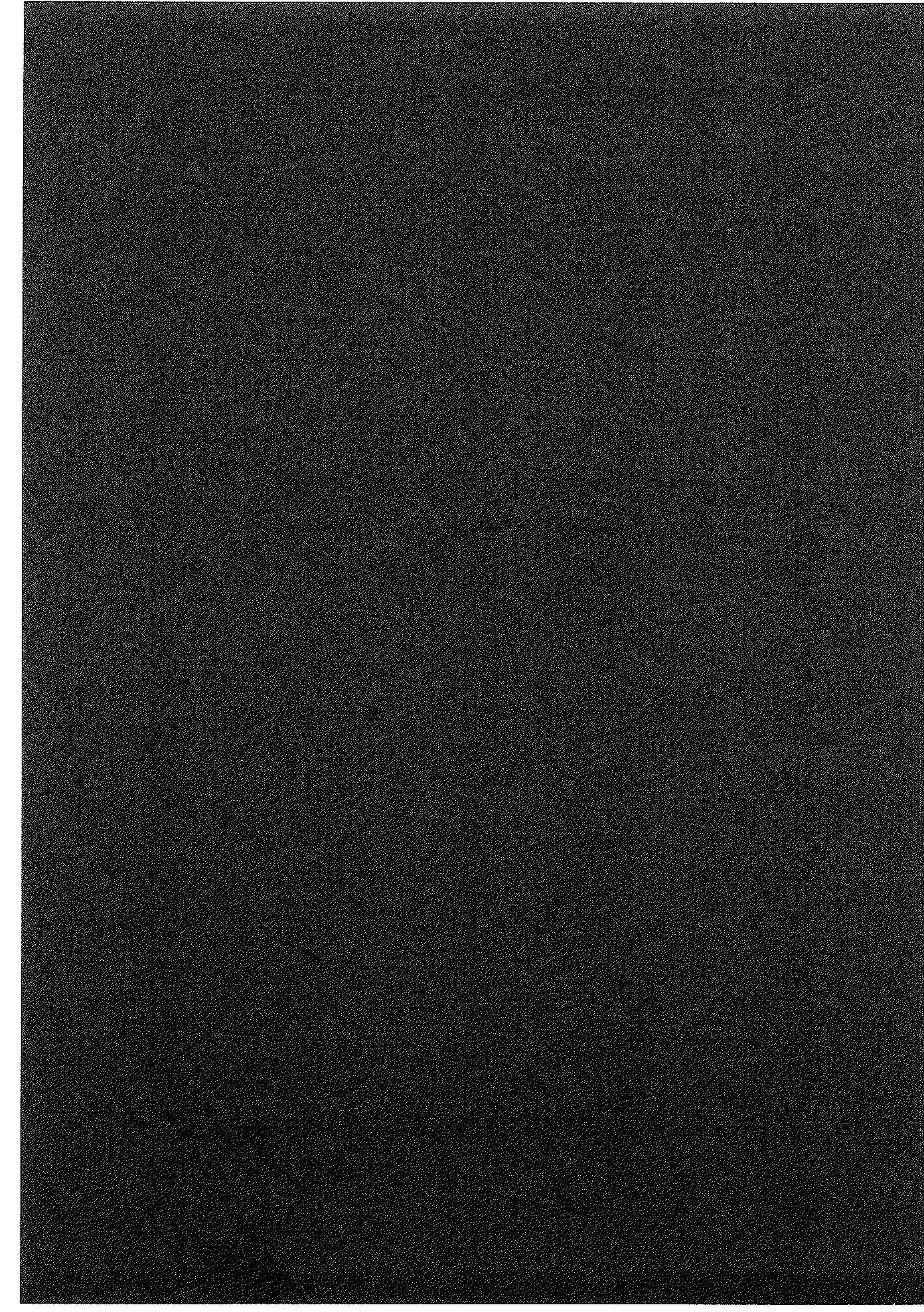
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ISOLATION AND STRUCTURE DETERMINATION OF ACTIVE COMPOUNDS FROM CENTAUREA SPECIES

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ISOLATION AND STRUCTURE DETERMINATION OF ACTIVE COMPOUNDS FROM CENTAUREA SPECIES*

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As a part of our continuing investigations of Turkish plants in order to establish their chemical properties, we have studied some *Centaurea* species.

In a previous study we investigated *Centaurea kotslochyi* which showed a slight activity on 3 PS test system (tests were conducted by NIH). From chloroform extract we have isolated four guainolide type sesquiterpene lactones, one of which was a new compound, and their structures were determined by spectral and chemical reactions. All compounds contained α-methylen-γ- lactone function as shown in Fig. 1[1].

Fig. 1

Since *Centaurea coronopifolia* showed promising activity against 3 PS test system we have decided to study this plant and obtain the active compounds. The aerial parts of *C. coronopifolia* was extracted with chloroform and chromatographed on a silica gel column and eluted with benzenechloroform by several proportions. The polarity was increased by gradual amounts of chloroform. Fractions, contain the same compounds, were combined and the single compounds were obtained by preparatif plates. Totally seven

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compounds were isolated. We have determined structure for four of these compounds up till now. As shown in Fig. 2, 3, 4, 5 all compounds are germacren type sesquiterpene lactones and have α-methylen -γ- lacone function, it is well known that this group is essential for cytotoxity. In several articles it has been reported that many sesquiterpene lactones possessing this group show cytotoxic activity[2,3,4].

Compound 1: IR spectra exibiting the following peaks cm^{-1} 3450 (OH), 1750 1150 (α, β -unsaturated -γ- lactone), 1710, 1275 (ester function), 1645 (C=C) indicated that the compound was a lactone and UV spectrum showed no conjugation by giving the absorption max. at 214 nm.

200 MHz ^1H NMR spectrum showed the characteristic duplets of α - methylen -γ- lactone group at 6.32 and 5.69 ppm (J = 3, 3.5 Hz), 1.24 ppm a singlet for a methyl adjacent to an oxygen and 1.83 ppm a singlet for a teritary methyl group. A duplet at 2.62 ppm J= 9 Hz is very characteristic for an epoxide proton in germacren type sesquiterpene lactones. In addition to these signals spectrum showed the other major peaks of the skeleton 4.32 dd (J= 6, 9.5 Hz), H – 6), 3.32 multiplet (H- 7), 4.58 (H- 1) and 5.30 m a proton bearing an ester group). In the other hand, 4.35s (br) and 4.48d (J = 6 Hz) indicated two CH_2 - O - groups and multiplet at 6.89 ppm for a vinylic proton in side-chain.

These data clearly show the side chain must be a derivative of tiglic acid and MS spectrum; indicating molecular peak at 378 and a fragment at 97 with the intensity of 98% (side chain-H_2O) confirmed the molecular formula as $C_{20} O_7 H_{26}$.

Fig. 2

Fig. 3

Compound 2: All spectroscopic data are very similar to compound 1 except ester function as side-chain. In 200 MHz ^1H NMR spectrum; 2.02 ppm a tertiary methyl singlet and at 5.99 ppm a vinylic proton multiplet show that the side chain must be senecioic acid derivative.

Compounds 3 and 4: IR and UV spectrum of these compounds are similar to those of compound 1 and compound 2, 400 MHz ^1H NMR spectrum shows additional hydroxyl group at C-9 position. A duplet at 4.57 ppm J = 4 Hz and a singlet (br) at 4.35 ppm indicate H- 8 (proton, geminal to the ester function) and H – 9 (proton, adjacent to an hydroxyl group) respectively. The upfield chemical shift of H – 8 and unusual splitting of H – 9 required more investigation, by spin-decoupling experiments, these protons were properly assigned.

Fig. 4

Fig. 5

Structure elucidation of the other compounds are still under investigations and will be discussed.

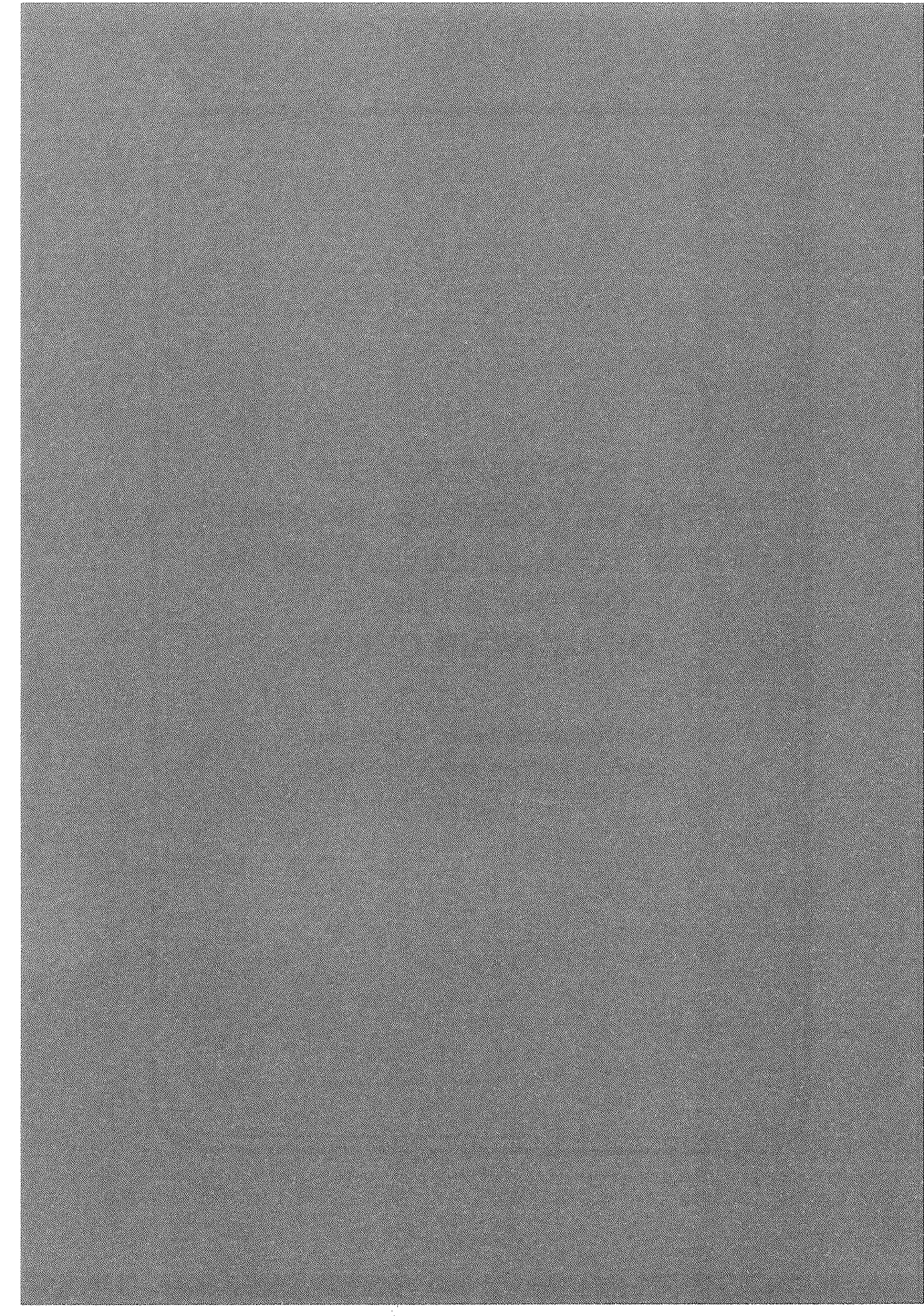
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CYTOTOXIC EFFECT OF THE GLYCOSIDES OBTAINED FROM ECBALLIUM ELATERIUM ON THE S-PHASE OF L-STRAIN CELLS

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CYTOTOXIC EFFECT OF THE GLYCOSIDES OBTAINED FROM ECBALLIUM ELATERIUM ON THE S-PHASE OF L-STRAIN CELLS*

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Ecballium elaterium is a well investigated plant which was tested against sarcoma 37 test system in 1952[1]. Elatericin A (1) and B (2) were isolated from this plant and their structures were elucidated[2-7].

(1)
elatericin A

(2)
elatericin B

Later a group of cucurbitacins (C, D,E, H, G, I, M) (3)[8], steroidal compound, elasterol (4)[9], a lignan, liqballinol (5)[10] and cucurbitacin A(6)[11] were obtained from the same plant.

Although most of of the cucurbitacins showed antitumor activity on KB cells, they showed negative or marginal *in vivo* activity against P388 leukemia, L1210 leukemia, B16 melanoma and Lewis lung tumor[12]. Cucurbitacins are found to be toxic compounds.

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R	R_1	R_2	R_3	
R = OH,H	R_1 = OAc	R_2 = CH_2OH	R_3 = OAc	Cucurbitacin C
R = 0	R_1 = H	R_2 = CH_3	R_3 = H	Cucurbitacin D
R = OH, H	R_1 = H	R_2 = CH_3	R_3 = H	Cucurbitacin F

In this country, the aqueous extract of the whole plant is being tested on volunteer patients, promising results were obtained in hermorrhoid, cancer, especially intestinal cancer patients. The extract is taken orally in extended periods. It was also claimed that the extract stops the symptoms of multiple sclerosis, although the nature of this latter sickness needs a long period of observation before coming to a conclusion. Since the aqueous extract was claimed to have shown these activities and no toxicity was observed until now, we decided to initiate a study on the aqueous extract of the whole plant. In order to find the active compound(s) the fractions and single compounds were first tested on cell cultures, later they will be tested on *in vivo* systems.

The tests are being conducted at the Faculty of Medicine (Çapa) as follows: L-strain cells were seeded on coverslips (4.3×10^4 cell per 20 mm) and put into petri dishes containing Medium 199, plus inactive calf serum (10%), 100g streptomycin and 100 IU penicillin. The petri dish was left at 37°, pH 7.2 in an atmosphere composed with 95% air and 5% CO_2 for 24 hours. Then the incubation media was replaced with the media containing the plant extracts or single compounds. Control groups were worked with incubation media which contains 1 Ci/ml ^{3}H-thymidine (TRA-120 Radiochemical Centre Anmersham). After 20 minutes the media were removed and washed with Hank's buffered salt solution and

fixed with Cornoy fluid. The cover slips fixed on slides using colourless nail polish. The slides then were covered with AR10 stripping film and left in the dark for 4 days. At the end of this period the films were developed and stained with Giemsa. The percentage of labelled and unlabelled cells were calculated.

The crude extract of the plant showed promising activity in reducing cell division in the S-phase. Therefore, this extract was fractioned on a Si-gel column, similar fractions were combined and each of the 8 fractions thus obtained, were tested as given above and the most active fraction was established. By using preparative TLC separation in addition to elatericin A and B we have obtained 7 glycosides. Each of these compounds were tested on L-strain cells, all of them showed some activity and the tests are still being carried out.

(4)
elasterol

(5)
ligballinol

(6)
cucurbitacin A

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AJMALINE IN THE MANAGEMENT OF CARDIAC ARRHYTHMIAS

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AJMALINE IN THE MANAGEMENT OF CARDIAC ARRHYTHMIAS*

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INTRODUCTION

Ajmaline (Gilurytgmal[R]) a tertiary indolin base was first isolated, from the Indian plant, *Rauwolfia serpentina* by Dr. Salimuzzaman Siddiqui[1], and named after Hakim Ajmal Khan, a pioneer of Tib in India, who had extensively used products of Rauwolfia as anti-hypertensive and cardiac sedative. Ajmaline is a member of a second group of Rauwolfia derivatives which have no sedative, hypnotic or hypotensive effects. It has been found to have potent anti-arrhythmic properties[2,10] and is extremely successful in the treatment or arrhythmias assoacited with Wolff-Parkinson-White syndrome[11-15].

This paper presents our ongoing experience with the drug and summarises the world literature.

MATERIALS AND METHODS

First Study

Our first study was carried out in England[8]. Ajmaline was administered intravenously (50 mg, slowly) and was effective in termination of ectopic tachycardia in 50% of cases. It also proved to be an effective prophylactic agent orally. In one case cardiac failure was aggravated after the intravenous injection.

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Current Study

Since ajmaline is still not available in Pakistan, the present study is being carried in a restricted manner. In the present ongoing study in 8 cases, 12 episodes of ectopic tachycardia have been studied. The series includes 9 males and 3 females, age range 32-65 years (mean 46 years). 7/9 (77%) episodes of supraventricular tachycardia (4/4 episodes of nodal tachycardia) and two out of three episodes (66%) of ventricular tachycardia were sinoverted with ajmaline 50 mg intravenously within 10-15 minutes after the injection. In one case transient asystole, responding to external massage was recorded. Some of these cases had received intravenous digoxin and pindolol.

DISCUSSION

Experimentally in dogs, electrophysiologic studies of ajmaline produced A-V interval and QRS prolongation, beneficial effects on digoxin toxicity, no significant effects on ventricular automaticity and decreased arrhythmias associated with ischemia[16]. Hemodynamically, ajmaline slows down pulse rate, atrial pressure and stroke volume in increasing doses. Ajmaline had a positive chronotropic effects on sinus node automaticity in conscious dogs, in contrast by no effect on ventricular automaticity[16]. This difference is explained by the fact that phase IV depolarisation in these two areas may be due to different electrophysiologic mechanisms[17].

Ajmaline significantly depresses intraventricular conduction as the main mechanism of action of anti-arrhythmic effect. Ajmaline injection leads to decrease in QRS amplitude in healthy subjects and patients[18]. Ajmaline has some sympatholytic activity[20], a negative ionotropic effect[21]. Electron microscopy with therapeutic ajmaline revealed signs of cellular stimulation in the heart muscle of guinea pigs[23].

Intravenous ajmaline (0.14 mg/kg body weight) in healthy persons produced alteration of the electric conductivity, decrease of stroke volume and cardiac output. Ajmaline also leads to decrease of pyruvate and lactate of venous blood[24] and should be used in cautious dosage in patients with liver damage[25]. Ajmaline is used, as a diagnostic tool, in blocking ventriculo-atrial conduction, without simultaneous effect on A-V conduction, in cases of ventricular tachycardia with retrograde conduction, of atria[26]. Toxic effects include hypotension, decreased cardiac output and atrioventricular block. Ajmaline is contradicted in atrial flutter and severe conduction disorders.

Quinidine, ajmaline and *beta* blockers are effective in eliminating atrial and ventricular ectopies which often initiate paraxysmal tachycardia in W-P-W syndrome.In a haemodynamic study of ajmaline, no significant haemodynamic effect was observed after 50 mg injection: QRS widened in all cases, bundle branch block occured in 3/11 cases who were also taking digoxin. This drug should be used with caution in digitalised patients[18]. Ajmaline increased refractory period of the accessory pathway, with temporary or complete block, lengthening of H-V interval and prevented initiation of tachycardia[14].

Ajmaline is an effective antiarrhythmic agent[11]; in experimental atrial fibrillation in dogs mortality was lowered to about 40-50% by propranolol, ajmaline and bretylium[27]. Kliensorge, *et al*[28] introduced this as an antiarrhymic agent in Europe. Antiarrhymic effects of ajmaline are due to prolongation of the refractory period of the heart, and due to a less pronounced slowing of conduction in atrial and the ventricular conduction system[29]. In an intraindividual comparative study in 15 patients with chronic stable ventricular extrasystoles of various origins, in the order of effectiveness were ajmaline, propafen and lidocaine and suppression of extrasystoles was most marked after ajmaline[10]. In our first stydy[8] and the

current study antiarrhythmic qualities of this drug have been confirmed.

In a series of 66 cases of paraxysmal supraventricular tachycardia sinoversion was obtained in 58 cases (88%)[33]. In 4 patients with 87 episodes of tachycardia, 85 episodes (96%) were sinoverted; 17/27 cases of atrial fibrillation and 4/7 cases of atrial flutter were also sinoverted. Ajmaline was ineffective in chronic atrial fibrillation, it abolished W-P-W syndrome in 17/27 cases. Serious side effects in this series were observed in 2/66 (3%) cases. In one case with bundle branch block a short asystole occurred and in the other transient ventricular flutter was observed[30]. Ajmaline has been recommended as a safe drug for management of arrhythmias in children intravenously and orally[31, 32]. Ajmaline has been effective in post-infarction tachycardia and should not be used in arrhythmias associated with halothane anesthesia [7, 33].

Ajmaline is found to shorten the action potential duration and refractory period in normal Purkinjee fibres. It has been postulated that ajmaline blocks anomalous bundle, but not conduction in the normal heart[34]. Ajmaline in 24 cases of pre-excitation syndrome lenghtened P-R interval in 85%, delta-wave disappeared in 64% and changes in QRS time in 58%[35]. In this study, effect of the drug on intraventricular and A-V conduction produced significant delays, requiring cautious use in cases with bundle branch block. In 35 cases of W-P-W syndrome, ajmaline intravenously caused temporary interruption of pre-excitation of 60% of cases[36]. It is recommended that athletes with W-P-W can compete in games, excepting those who also have paraxysmal tachycardia.

In another series of W-P-W syndrome ajmaline produced P-R interval prolongation, and most striking influence was H-V prolongation, appearing within 30-60 seconds of administration and lasting for 15-60 minutes[15]. In this series rapid atrial and

ventricular pacing following ajmaline confirmed complete blockade of anomalous pathways.

Rarely ajmaline has been used suicidally by over-dosage [37, 38]. A method has been reported for identification and quantification of ajmaline in autopsy material in cases of suspected suicidal attempts by ajmaline[39].

CONCLUSION

Ajmaline has now been effectively used for half a century. The major work emanates from the German centres and most of the literature is in the German language. It is an effective antiarrhythmic agent and is particularly effective in arrhythmias associated with Wolff-Parkison – White syndrome.

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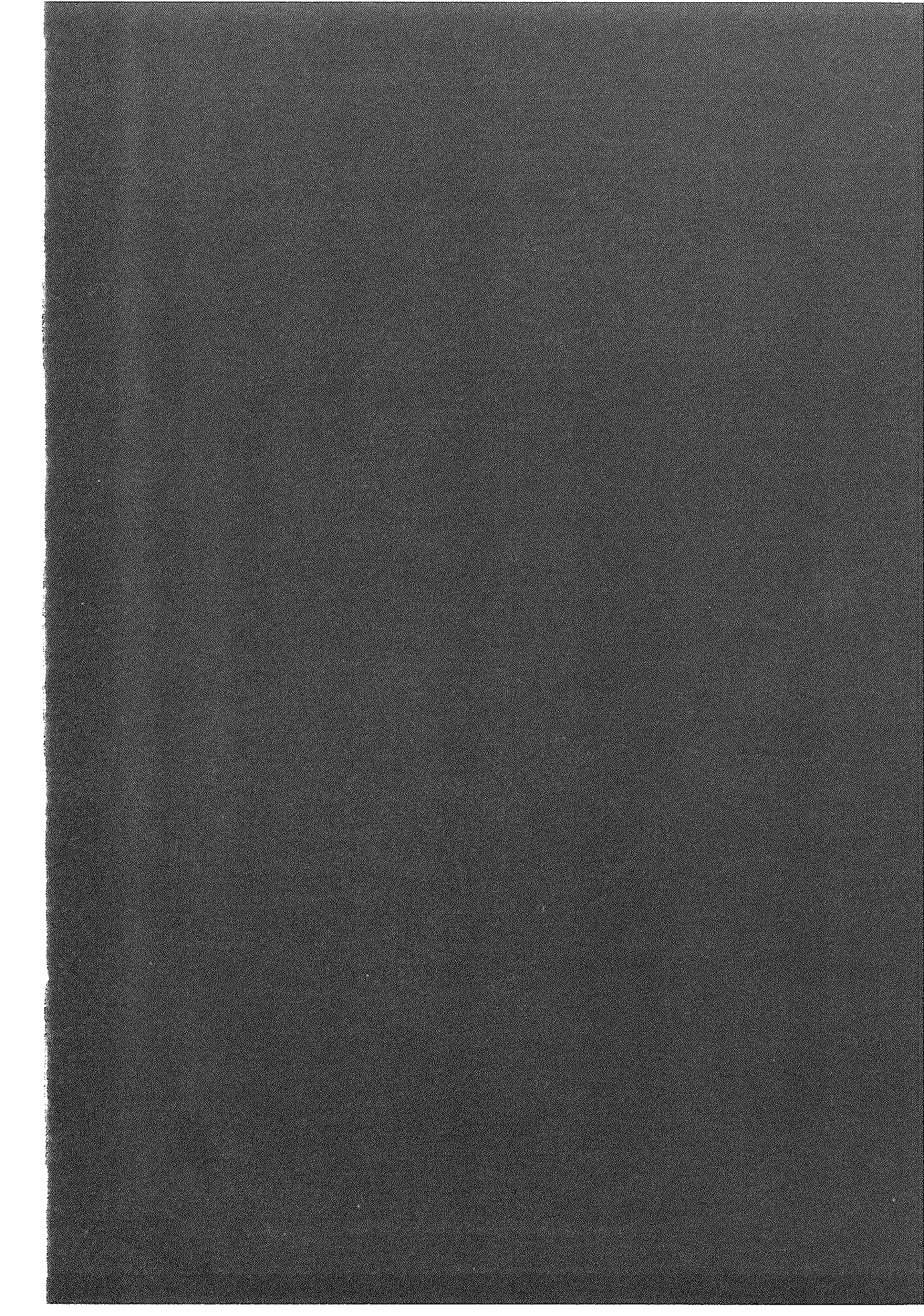
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SOME RECENT ISOLATION AND SYNTHETIC STUDIES ON THE CONSTITUENTS OF INDIGENOUS MEDICINAL PLANTS

Drs. Atta-Ur-Rahman, G.A. Miana, Y. Ahmad, M.A. Khan, V.U. Ahmad, F. Zehra, A.A. Ansari, M. Bashir, M. Sultana, I. Hasan, Mehrun Nisa, S. Farhi, T. Zamir, M. Shamma, G. Blasko, N. Munugesan, J. Clardy, A.J. Freyer, S. A. Drexler, Wolfgang Voelter and P.W. Le Quesne.

PAKISTAN



SOME RECENT ISOLATION AND SYNTHETIC STUDIES ON THE CONSTITUENTS OF INDIGENOUS MEDICINAL PLANTS*

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PAKISTAN

The bulk of the population of the Afro-Asian countries, particularly those living in villages, rely on the indigenous medical system to provide relief from disease. Systematic scientific investigations, particularly during the current century, have resulted in the identification of a growing number of active constituents many of which are now routinely used in modern medicine. These include reserpine for the treatment of cardiac arrhythmias, vincamine as a vasodilator, and vinblastine and vincristine as anti-tumour agents, etc. Isolation, structural and synthetic studies have accordingly been directed in many laboratories around the world, including ours, to isolating new natural products which could prove to be valuable chemotherapeutic agents. Some of the recent studies carried out by my group at Karachi are briefly presented here.

ISOLATION AND STRUCTURAL STUDIES ON BERBERIS ARISTATA

Berberis aristata DC (*Berberidaceae*) is a shrub found in the northern mountainous regions of Pakistan and India as well as in the Nilgiri Hills of Southern India. The extracts, made from the

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root bark are known as "resaut" and are used in the traditional system of medicine for the treatment of jaundice and skin diseases. As a result of careful isolation studies, 2 new alkaloids, "Karachine" (1) and "Texelamine" (2) have recently been isolated (G. Blasko, *et al*, 1982a, G. Blasko, *et al*, 1982b). Karachine is the first naturally occurring berbinoid of this skeletal system and is the most complex of more than 50 protoberberine alkaloids presently known. Its structure (1) has been elucidated largely on the basis of

its high resolution mass and 360 MHz (FT) NMR spectra, and the positioning of groups confirmed by Nuclear Overhauser Effect studies.

The UV spectrum of Karachine, λmax EtOH 226 and 285 nm (Log ε 3.90 and 3.62), was suggestive of a tetrahydroprotoberberine. The mass spectrum shows the molecular ion at m/e 443, and the base peak at m/e 336. The latter peak fits exactly for the molecular ion of berberine or epiberberine and is formed by loss of 97 mass units from the molecular ion via cleavage alpha to the nitrogen atom (C-14 to C-ε bond), followed by a retro-Diels-Alder process. The m/e 97 fragment corresponds to C_6H_9O, or, more specifically, to 2 moles of acetone minus the elements of water (3). A sharp absorption band at 1710 cm^{-1} in the IR spectrum ($CHCl_3$) denoted the presence of a non-conjugated carbonyl.

The 360 MHz (FT) NMR spectrum in $CDCl_3$ presented a complex pattern, but allowed for the tentative assignment of expression 1 to Karachine.

In order to settle conclusively the nature of the substitution pattern in aromatic-rings A and D, an n.o.e. study was carried out. Irradiation of the C-10 methoxyl singlet at *delta* 3.77 resulted in an overall 11.6% increase in the area of the δ6.52 and δ6.55 ring D aromatic doublet of doublets. Alternatively, irradiation of the H-1 singlet at δ6.73 gave a 2.89% increase of the δ2.70 and 2.72 doublet of doublets assigned to the C-ε protons, as well as to 5.6% increase of the singlet at δ3.07 due to H-13. Significantly, irradiation of either the H-1 or H-4 singlets at δ6.73 and δ6.17 respectively led to no observable n.o.e. for the methoxyl absorptions. Further support for the structure of Karachine has come from its borohydride reduction, and analysis of the mass and NMR spectra of the corresponding alcohol.

Karachine must arise by the condensation of berberine (3) with 2 moles of acetone and accompanying loss of water, as suggested in

the Scheme (1). It is the first naturally occurring berbinoid incorporating acetone units (Govindachari, *et al*, 1981). It is a true alkaloid and not an artefact of isolation since (a) optically active, as well as inactive, naturally occurring adducts of the related benzo-phenanthridine alkaloids with acetone are known (Shamma, *et al*, 1972), (b) no acetone was used during the isolation process, and (c) various attemps on our part to obtain Karachine by condensation of berberine with acetone at varying pHs were to no avail.

Texilamine (2) was isolated by chromatography of the alkaloidal fraction (8 g) using neutral alumina. Besides a consistent UV spectrum, the 360 MHz (FT, $CDCl_3$) NMR spectrum of taxilamine

shows H-5 and H-8 as singlets at δ7.15 and δ7.40, respectively; H-3 and H-4 as a doublet of doublets at δ8.46 and δ7.66 J_{vic} = 5.5 Hz); and H-5′ and H-6′ as another doublet of doublets at δ6.44 and δ6.28 (J_{vic} – 9.1 Hz). The 4 methoxyl signals appear as singlets at δ3.92, 3.96, 3.97 and 4.06. This spectrum bears a distinct resemblance to that reported for ugosinone (Wu, *et al*, 1980). The mass spectrum of taxilamine confirmed the molecular formulation $C_{20}H_{19}O$ N and the structure (2) assigned.

Taxilamine (2) is the fourth member of its class of pseudobenzyl isoquinoline alkaloids and must probably have been formed in nature through oxidative rearrangement of palmatine to suply initially polycarpine. Hydrolytic N-deformylation followed by further oxidation would then afford taxilamine (Murugesan, *et al*, 1979).

ISOLATION AND STRUCTURAL STUDIES ON THE CHEMICAL CONSTITUENTS OF FAGONIA INDICA

Fagonia indica Linn. is a small spiny undershrub which is widely distributed in Pakistan. An aqueous decoction of the leaves and young twigs is a popular remedy for cancer in its early stages. A new sapogenin "Nahagenin" (5) (Atta-ur-Rahman, *et al*, 1982a) has been isolated from the hydrolysed extracts of the aerial parts of the plant, and its structure has been elucidated on the basis of a 100 MHz NMR spectrum, a 100 MHz CMR spectrum and high resolution mass spectrum.

The substance analyzed for $C_{30}H_{48}O_4$ (confirmed by high resolution mass spectrometry, m/z = 472.3740 mass, 472.3552 for $C_{30}H_{48}O_4$). Major peaks in the MS occurred at m/z 454, 436, 424, 409, 395 and 261. The IR spectrum ($CHCl_3$) showed peaks at 1740 cm^{-1} and 3460 cm^{-1} suggesting a δ-lactone and hydroxy groups. The substance readily afforded a diacetate (m/e = 556), but was found to be remarkably inert to attempted hydrolysis of the lactone. The ^1H NMR showed no olefinic protons. The ^{13}C NNR recorded on a

400 HHz instrument confirmed the presence of 30 carbons. The carbon atoms in the A and B rings were readily recognised by comparison with corresponding signals of known pentacyclic triterpenoids (Knight, *et al,* 1974). Eight quaternary centres and 6 methyl groups were also identified. The ^{13}C NMR displayed a resonance at δ177.29 for the carbonyl carbon, and 3 resonances at δ84.72 (s), 76.54 (dd) and 71.92 (d) for the oxygen-bearing carbons C (20), C(3) and C(23) respectively. On the basis of these spectral data, structure (5) was assigned to nahagenin which has been confirmed by an unambiguous structure determination by a single crystal X-ray diffraction analysis carried out by Prof. Clardy and co-workers at Cornell University.

ISOLATION AND STRUCTURAL STUDIES ON BUXUS PAPILOSA

Buxus papilosa (*Buxaceae*) is a shrub which occurs abundantly in the northern regions of Pakistan. Extracts of *Buxus* species have been used since ancient times for the treatment of a wide variety of diseases including malaria and venereal disease. *Buxus papilosa* has found use in the indigenous system of medicine as a febrifuge for relief of rheumatism and for the treatment of a number of other ailments. Four new alkaloids, papilamine (6) (Atta-ur-Rahman, *et al,* 1983a), papilicine (7) (Atta-ur-Rahman, *et al,* 1983b), moenjodaramine (8) (Atta-ur-Rahman, *et al,* 1983c), and harappamine (Atta-ur-Rahman, *et al,* 1983d), have recently been isolated by us from the leaves of this plant and their structures elucidated on the basis of the spectral data of the alkaloids as well as their derivatives. The spectral data obtained for each alkaloid are given against each structure.

The UV spectrum of moenjodaramine showed absorption maxima at 207, 237, 245 and 254 nm, characteristic of the presence of a 9 (10→19) *abeo*-diene system (Khuong, *et al,* 1966). An identical UV spectrum is encountered in buxamine E, buxaminol E and papilamine (Atta-ur-Rahman, *et al,* 1983a). The proton NMR spectrum ($CDCl_3$) showed 3 singlets, corresponding to the 3 tertiary

methyl groups at δ0.71, δ0.75 and δ1.03. The secondary (C-21) methyl group resonated as the doublet at δ0.88 (J= 6 Hz). A 3-proton singlet resonating at δ2.1, was assigned to the – NCH_3 group, while another peak resonating at δ2.2 and integrating for 6 protons was assigned to the – $N(CH_3)_2$ group attached to C-20. A set of AB doublets resonating at δ3.24 and ε3.82 was assigned to C-29 methylene protons α - to the C-3 nitrogen. A singlet at δ5.98 was ascribed to the isolated olefinic proton at C-19 while a multiplet centred at δ5.55 was assigned to the C-11 olefinic proton.

(20) (21) (22) (23) (24) (25)

The mass spectrum of the compound afforded the molecular ion at m/z = 426.3609 which corresponded to the formula

$C_{28}H_{46}N_2O$ (calcd. 426.3609). The substance showed a base peak at m/z 58.0650 corresponding to the composition $C_3H_8N^+$ which suggested the loss of CH_2N^+ $(CH_3)_2$ characteristically encountered in alkaloids bearing a – $N(CH_3)_2$ grouping on ring A, and which may be formed in moenjodaramine by intramolecular proton transfer and cleavage. Another peak at m/z 57.062 corresponded to the fragment $CH_2 = {}^+N(CH_2)CH_3$. A peak at m/z 85.0883 was in accordance with the composition $C_5H_{11}N$ (calc. 85.089) which was attributed to $(CH_2)_2CH = {}^+N(CH_3)_2$ formed by the cleavage of ring A along with the side chain. A peak at m/z 72.0810 having the composition $C_4H_{10}N^+$ corresponded to the loss of $CH_3,CH = N^+(CH_3)_2$ commonly encountered in alkaloids bearing a- $CH(CH_3) - N(CH_3)_2$ grouping on ring D (Waller, *et al.* 1980). Another peak at m/z = 71.0734 having formula $C_4H_9N^+$ was assigned to the fragment $CH_2 - CH = N^+ (CH_3)_2$ formed by cleavage of ring A along with the side chain.

In the light of the above studies, structure (8) has been assigned to moenjodaramine. This substance has previously been reported as a synthetic product prepared from desoxy-16-buxidienine C (Khuong, *et al.* 1971), but it has not been isolated. A second alkaloid, harappamine was similarly established to have structure (9).

Moenjodaramine (8) and harappamine (9) are the first reprsentative members of a new class of pentacyclic natural products bearing both a tetrahydrooxazine ring and a 9 (10→19) *abeo*-diene system.

ISOLATION AND STRUCTURAL STUDIES ON THE CHEMICAL CONSTITUENTS OF CATHARANTHUS ROSEUS

Studies on the alkaloids of *Catharanthus roseus* have resulted in the isolation of a new alkaloid, to which structure (10) has been assigned. The substance afforded a UV spectrum which was typical of a dihydroindole system, showing absorption maxima at 212, 246 and 303 nm and minima at 276, 226 nm. The IR spectrum showed the presence of an ester carbonyl absorption at 1730 cm^{-1}. The mass

spectrum was very similar to that reported for vindolinine (Djerassi, *et al*, 1962) and 19-epi-vindolinine (Mehri, *et al*, 1972). A high resolution mass measurement on the molecular ion afforded the exact mass to be m/z 336. 1837 in agreement with the formula $C_{21}H_{24}N_2O_2$. The C-13 NMR spectrum of the alkaloid (10) (broad-band and off-resonance) showed interesting similarities to the C-13 NMR spectra reported for 19-R-vindoline (Ahond, *et al*, 1974), 19-S-vindoline (Ahond *et al*, 1974), and 16-epi-19-R-vindolinine (Ahond, *et al*, 1974). The ester carbonyl carbon resonated at δ173.47,whereas the methyl of the ester group resonated at δ52.6 (quartet). The substance afforded 4 doublets for the tertiary aromatic carbons, and 2 singlets for the 2 quaternary carbon atoms. A characteristic singlet appeared at δ81.36 corresponding to the quaternary carbon atom α to the indoline nitrogen (Atta-ur-Rahman, *et al*, 1983e).

The H-NMR spectrum of (10) recorded on a 200 MHz instrument showed the presence of a doublet at δ0.62 (J = 7.4 Hz) which is assigned to the C-18 methyl protons. The proton adjacent to the carbomethoxyl function resonated as a double doublet at 3.18 (J_1 = 12.2 Hz, J_2 = 5.8 Hz). A double-doublet at δ6.41 was assigned to the olefinic proton at C-15, showing coupling with the vicinal olefinic proton and an allylic coupling with the C-3 proton (J_1 = 10 Hz, J_2 = 2.8 Hz). The other olefinic proton at C-14 resonated as a doublet of double doublets at δ5.84 (J_1 = 10 Hz, J_2 = 5.2 Hz, J_3 = 1.8 Hz). The chemical shift of δ0.62 for the methyl group is consistent with a 19-S - configuration as the methyl group of 19-S-vindolinine resonates at δ0.57 while the methyl group in 19-R-vindolinine resonates at δ0.95.

Direct TLC comparison with authentic samples of vindolinine and epivindolinene showed that the substance could be just separated from these 2 materials in 25% ethanol in ehtylacetate on a silica gel plate. In order to confirm the structure, the alkaloid (10) was subjected to an oxidative cleavage reaction (Janot, *et al*, 1962, Rasoanaivo, *et al*, 1974) with iodine/THF/H_2O/Na_2CO_3 when it was found to be smoothly converted to the iodo compound (11). On hydrogenolysis with Raney Ni at 30°C for 2 h, the iodo compound was found to be transformed to (-) –vincadifformine (12). When the same hydrogenolysis experiment was repeated at 0°C for 5 min.

quantitative conversion to tabersonine (13) was observed (Scheme 2). The identity of the synthetic hydrogenolysis products was established by direct chromatographic and spectroscopic comparison with authentic samples of tabersonine and vincadifformine.

16-epi-19-S-vindolinine, when refluxed in benzene for 3 h in the presence of an equimolar amount of lead tetraacetate, was found to be smoothly transformed to 2 faster running products. The major product formed in 70% yield afforded a normal indolic U.V. spectrum. The I.R. spectrum (KBr) showed bands at 1655 cm^{-1} and 1730 cm^{-1}, which were assigned to N_b-CHO and $-CO_2CH_3$ groups respectively. The mass spectrum showed M^+ at 352.1783 (calc. for $C_{21}H_{24}N_2O_3$, 352.1786), and other major peaks at 320, 293, 214, 169 and 154. The PMR spectrum ($CDCl_3$) showed resonances at δ1.23, (3H, d, J = 5.6Hz, C = CH-CH_3), δ3.67 (3H, s, OCH_3), δ5.46 (IH, q, J = 5.6 Hz, C = CH-CH_3), δ5.7-6.1 (2H, m, HC=CH), δ7.6 -6.9 (4H, m. aromatic), δ8.00 (1H, s, N_b -CHO) and δ8.35 (IH, s, NH). Irradiaton at δ5.46 resulted in the collapse of the methyl group at δ1.23 to a singlet.

(10)

Na_2CO_3/H_2O | I_2, THF

(11)

Raney Ni/H (rt) / Raney Ni/H (30°C)

(13) (12)

The above spectroscopic data were identical with those for (14), a prdouct previously reported to be formed from 19-iodo-tabersonine on heating with sodium acetate in run on DMF (Diatta, *et al.* 1976). In order to confirm the structure of the oxidation product, 16-epi-19-S-vindolinine (10) was oxidized with iodine under conditions previously described for the oxidation of its diastereo isomer (Rasoanaivo, *et al*, 1974b). This afforded the corresponding 19-iodo-tabersonine in quantitative yields. Treatment of the latter with sodium acetate in hot DMF afforded (14). A direct spectroscopic and chromatographic comparison of the product formed by lead tetraacetate oxidation with that prepared from 19-iodotabersonine (Diatta, *et al*, 1976) unambiguously established its structure. A plausible mechanism for the formation of (14) is pesented in (Scheme 3).

The second minor product formed in the lead tetraacetate oxidation possessed a U.V. characteristic for the dihydroindole system. Further work on the structure of this material is under progress.

The facile formation of (14) from (10) is biogenetically interesting particularly in view of the occurrence of the binary indole alkaloids such as catharine (16) (Rasoanaivo, *et al*, 1974c) in which one of the moieties bears a distinct resemblance to (14) and raises the interesting possibility that the indole moiety of catharine may arise by a parallel process occurring in a binary precursor alkaloid such as (15) (Scheme 4).

(15)

(16): R = 10-vindolinyl

A RAPID PROCEDURE FOR THE ISOLATION OF CATHARANTHINE, VINDOLINE AND VINBLASTINE

The binary indole alkaloids vinblastine (17) and vincristine (18) are among the most potent chemotherapeutic agents known to man, and are being used for the treatment of several different type of cancer including Hodgkins disease, acute leukaemia in children and choriocarcinoma. As both alkaloids occur only in minute traces

in the leaves of *Catharanthus roseus*, they cost several thousand dollars per gram. This has attracted the attention of a number of groups towards their synthesis.

vindoline

1) $NaBH_4$
2) $(CH_3CO)_2O$

vinblastine (17) R = CH_3
vincristine (18) R = CHO

Our earlier efforts in this field (Atta-ur-Rahman, *et al*, 1980) have led to 2 different syntheses of vinblastine based on functionalisation of the olefinic bond of catharanthine before (Atta-ur-Rahman, *et al*, 1976) (Scheme 5), or after (Atta-ur-Rahman, *et al*, 1978) (Scheme 6) (Atta-ur-Rahman, 1980b, 1981) coupling with vindoline. These syntheses employed a novel modification of the

Polonovski reaction developed by Potier and co-workers (Potier, *et al*, 1975). A similar approach to vinblastine has also been reported by the French group (Managaney, *et al*, 1979)

$H_2/Pd\text{-}C$

T.F.A.
Polonovski

$Tl(OAc)_3$

R=vindoline

R=vindoline

OsO_4
$NaBH_4$

(17):R=vindoline

R=vindoline

As the above synthetic routes were all based on catharathine and vindoline as starting materials, it was important to develop an isolation procedure which could afford these alkaloids in bulk without having to resort to extensive chromatography. We have now developed a rapid isolation procedure for the isolation of catharanthine, vindoline and vinblastine. This procedure has been tested and found to be extremely satisfactory both at laboratory and pilot-plant levels. This should produce catharanthine, vindoline and vinblastine much more readily, as well as stimulating further research on the development of new antitumoural derivatives of these oncolytic alkaloids. The procedure that we have developed (Atta-ur-Rahman, *et al*, 1982b, Atta-ur-Rahman, *et al*,

1983f), involves extraction of the alkaloids with pH 3 phosphate buffer, selective precipitations by use of appropriate solvents and selective extractions. For vinblastine, a direct and simple isolation procedure has been developed which does not involve any chromatography, but affords pure vinblastine sulphate in yields of 0.02% to 0.025% by weight of the dried leaves (8-10 gm of vinblastine from 40 kg of leaves). Pilot plant investigations have proved very successful and commercialisation of the procedure is under active exploration.

ISOLATION AND STRUCTURAL STUDIES ON THE CHEMICAL CONSTITUENTS OF BETULA UTILIS

Betula utilis is a tree commonly found at high altitudes in the temperate Himalayas extending from Chitral eastwards to Azad Kashmir, and in Sikkim and Bhutan. The infusion of its bark has found wide use in indigenous medicine as an antiseptic, carminative, and for hysteria. Our interest in the systematic investigation of the chemical constituents of Pakistani medicinal plants has led us to a chemical investigation of the bark of *Betula utilis*. This has resulted in the isolation of a new triterpenoid, "Karachic acid" (19), the structure of which has been solved on the basis of chemical and spectroscopic studies (Atta-ur-Rahman and Khan, 1975).

ISOLATION AND STRUCTURAL STUDIES ON THE CHEMICAL CONSTITUENTS OF CUCUMIS PROPHETARUM

The isolation of a number of cucurbitacins with cytotoxic properties prompted us to investigate the active principles present in the fruits of *Cucumis prophetarum* (*Cucurbitaceae*), a plant locally known as "Choti indrayan" or "Khar indrayan". It is a perennial trailing herb with ellipsoidal echinate fruits. The plant grows wild in various regions of Pakistan, Rajputana (India), Saudi Arabia and tropical Africa. The fruits are used in indigenous medicine as an emetic and purgative. It is known to contain cucurbitacins B and C and traces of cucurbitacins G and H.

Butyraldehyde and morpholine were condensed by the method of Stork (Stork, *et al*, 1963) to afford the morpholinoenamine (27). Alkylation of (27) with methyl acrylate gave the dialkylated product (28) which was hydrolysed with aq. acetic acid to give (29) in 45% yield.

Condensation of (29) with tryptamine afforded a gum which on purification through a silica column gave a mixture of 2 diaster-oisomeric compounds, which could readily be separated by preparative layer chromatography as (30) and (31) (Scheme 7), each of which crystallised as white needles m.p. 185-186°C and 230°C respectively.

Lithium aluminium hydride reduction of the diastereoisomeric mixture of (30) and (31) afforded the amine (26) in 20% overall yield; (26) is convertible to vincamine and eburnamonine by one of several routes (Kuehne, 1964; Wenkert and Wickberg, 1965; Hermann, *et al*, 1979). This, thus formally, constitutes a total synthesis of these alkaloids (Atta-ur-Rahman and Sultana, 1982c)

A TOTAL SYNTHESIS OF N-METHYL SECODINE

The currently accepted biosynthetic route to the indole alkaloids envisages the mediation of 14, 21-dehydrosecodine (34) which can undergo intramolecular Diels-Alder reactions in different ways to afford the Iboga alkaloids catharanthine (35), or the Aspidosperma alkaloid tabersonine (Wenkert, 1962). In spite of intensive efforts by several groups, the synthesis of dehydrosecodine has still not been accomplished because of its high susceptibility to oxidation, dimerization and polymerisation; the synthesis of N-benzyldehydrosecodine has recently been reported (Kutney, *et al*, 1982). Secodine has, however, been synthesised (Kuehne, *et al*, 1978, Kutney, 1979, Raucher, *et al*, 1981, Marazano, *et al*, 1977), and a number of approaches to the indole alkaloids involving the intermediacy of the secodine system have been studied (Kuehne, *et al*, 1979, Scott, *et al*, 1974). We have recently developed a short and high yield synthesis of N-methyl secodine based on a facile Friedel-Crafts acylation reaction at the indole 2-position (Atta-ur-Rahman, *et al*, 1983h) which is shown in Scheme 8.

N-methyl secodine (40) when refluxed in acetonitrile for 8h afforded the 2-hydroxy carbazole (42) as a major product (yield 80%). The facile conversion of the secodine derivative to the carbazole system (43) (Scheme 8) suggests the intermediacy of N-methyl dehydro-secodine (41) in the reaction which may have been formed through aerial oxidation. The generation of carbazole derivatives has previously been reported (Kutney, *et al*, 1982b, Scott and Cherry, 1969), and it has been proposed that 2-hydroxy

carbazole if formed via dehydro-secodine by an intramolecular rearrangement and hydrogen transfer mechanism. A parallel project aimed at synthesising N_a-benzylsecodine has also been carried out. This synthesis represents the shortest route (reported to date) to the secodine system. Attemps are presently underway to generate the corresponding dehydrosecodines for biomimetic transformations to the Aspidosperma and Iboga alkaloids.

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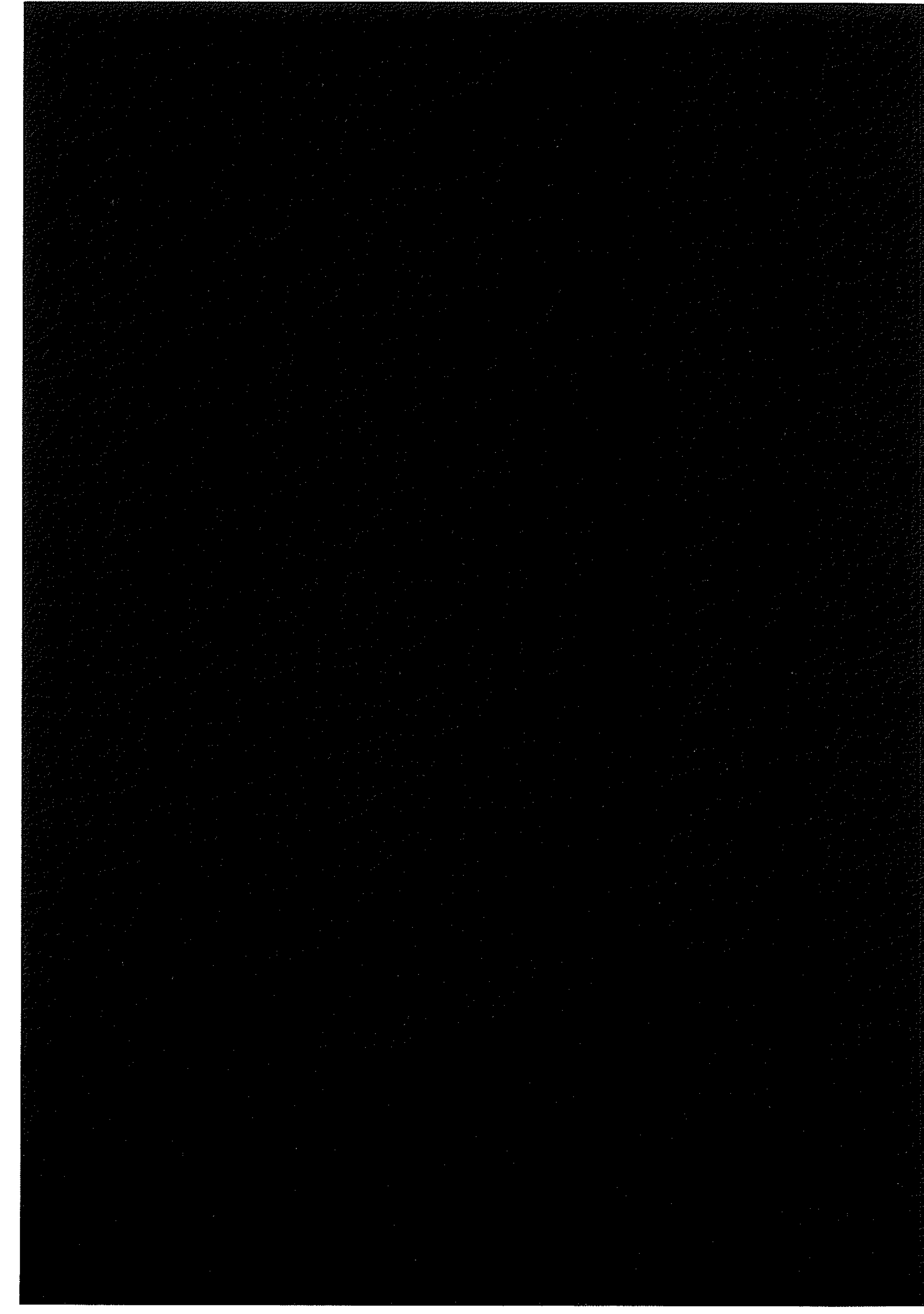
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STUDIES ON NEW INDOLE ALKALOIDS FROM MEDICINAL PLANTS

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STUDIES ON NEW INDOLE ALKALOIDS FROM MEDICINAL PLANTS*

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INTRODUCTION

Continuing investigations carried out on various plants in our laboratories have resulted in the isolation of forty-six new and a large number of known alkaloids. Their structures and configurations were determined with the help of modern spectroscopic techniques including 2D NMR (COSY-45, 2D J-resolved, NOESY), spin-spin decoupling, NOE difference measurements, hetero-COSY and ^{13}C-NMR (DEPT and GASPE) experiments[1-5].

(A) NEW INDOLE ALKALOIDS FROM RHAZYA STRICTA

Rhazya stricta Decaisne (*Apocynaceae*)[6] is a small, glabrous, erect, shrub which is widely distributed in Western Asia and abundantly found in Pakistan. It has long been used in the indigenous system of medicine for the treatment of various ailments[5-9]. The anti-cancer activity of some of the indole alkaloids of this plant is also reported[9-12].

The ethanolic extract of *Rhazya stricta* collected from a small village about 90 km from Karachi, was evaporated into gum, and the separation of crude alkaloids on the basis of their differential basicities have resulted in several pH fractions. These fractions were subjected to column and thin-layer chromatography to afford the following new alkaloids.

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Aspidospermidose (1)[13]

This new alkaloidal glycoside showed the UV absorptions (MeOH) at 211, 250 and 303 nm, characteristic for the dihydroindole chromophore. The IR (KBr) spectrum showed intense absorptions at 3400-3450 cm^{-1} (O-H), 1740 cm^{-1} (ketonic C = O), 1650 cm^{-1} (C = C), and 1050 cm^{-1} (C-O).

The high resolution mass spectrum of the alkaloid showed the molecular ion at m/z 442.2444, corresponding to the molecular formula $C_{25}H_{34}N_2O_5$, indicating ten double bond equivalents in the molecule. The peak at m/z 281.1978 ($C_{19}H_{25}N_2$) corresponded to the loss of 61 m.u. ($C_6H_9O_5$, a sugar unit) from the molecular ion while the highly oxygenated fragment at m/z 290.1016 ($C_{15}H_{16}NO_5$) suggested the attachment of the glycosidic linkage with the indole part of the molecule. When the compound was treated with D_2O and the mass spectrum recorded, the M^+ was found to be shifted by 3 m.u., thereby suggesting the presence of three exchangable hydrogen atoms. Linked scan measurements of metastable transitions established that the ion with m/z 290 arose from the ions at m/z 442 and 414 but did not fragment to the ions at m/z 210 and 124, suggesting that m/z 290 is independent of the aspidosperma part of the molecule.

The ^{1}H-NMR ($CDCl_3$, 300 MHz) spectrum showed a three-proton triplet at δ0.62 for the C-18 methyl protons. The C-19 protons appeared at δ1.54 and 0.89 as a multiplet, indicating their non-equivalence. The upfield chemical shift of the C-21 proton (δ2.34) suggested α-stereochemistry. The C-2 proton appeared at δ3.84 as a double doublet, the rather upfield chemical shift suggesting β-stereochemistry. The upfield chemical shift value of the C-12 proton (δ6.50) indicated the presence of an N_a-glycoside linkage. The presence of $C_6H_9O_5$ sugar unit was also evident from the NMR spectrum. The C-1′ proton appeared at δ4.91 as a broad singlet, the chemical shift being consistent with this type of system.

The C-5′ proton appeared at δ3.94, its downfield chemical shift reflecting the presence of the carbonyl function α-to this proton.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz), showed the presence of 25 carbon atoms. The multiplicity assignments were made by carrying out GASPE experiments. The C-18 methyl carbon resonated at δ6.84 while the C-19 appeared at δ29.32. The signal at δ66.32 was assigned to C-21, its chemical shift reflecting α-stereochemistry. Carbon-3′ which is attached to OH and carbonyl group resonated at δ80.41. The C-1′ carbon resonated at δ80.61, its chemical shift suggesting the presence of an N-glycoside linkage (rather than an O-glycosidic linkage) to the hetero-aromatic aglycone. The ^{13}C-NMR assignments are shown around structure 1.

The relative stereochemistry was determined by a series of NOE difference measurements. The two dimensional spectra (2D J-resolved, COSY-45°, NOESY) and ^{13}C-NMR experiments agreed with the proposed structure. On the basis of above data, structure 1 was proposed for aspidospermidose.

Bharhingine(2)[14]

This new strychnos-type alkaloid showed the UV spectrum (MeOH) with absorptions at 210, 230, 270, 307 and 327 nm. The IR spectrum ($CHCl_3$) afforded intense absorptions at 1715 cm^{-1} (amide carbonyl) and 2880-2920 cm^{-1} (C-H).

The high resolution mass spectrum afforded the molecular ion at m/z 292.1571, consistent with the molecular formula $C_{19}H_{20}N_2O$, indicating eleven double bond equivalents in the molecule. The peak at m/z 277 suggested the loss of 15 m.u. (methyl) from the molecular ion while the prominent peak at m/z 263 resulted due to the loss of the formyl group.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed the presence of ethylidene side chain. The C-16 proton resonated at δ4.64 as a multiplet. A doublet at δ8.55 ($J_{9,10}$ = 7.9 Hz) was assigned

to the C-9 proton, the downfield chemical shift providing strong indication of the presence of an N-formyl function. The C-12 proton resonated at δ 8.08 as a doublet ($J_{12,11}$ = 7.6 Hz). The rather downfield chemical shifts of the C-10, C-11 and C-12 protons are consistent with the presence of an N-formyl function. A singlet at δ 8.62 was assigned to the formyl proton, its upfield shift agreeing with the attachment of the formyl group to a nitrogen function.

Two dimensional ^{1}H-NMR measurements (2D J-resolved, COSY 45°) afforded data consistent with the proposed structure (3) for bharhingine. The NOE interactions between C-18H and C-21H established 'Z' configuration of the ethylidine side chain. On the basis of above data structure 2 was proposed for bharhingine.

Bisstrictidine (3)[15]

This new alkaloid was isolated as an amorphous material. Its UV spectrum (MeOH) showed absorption maxima at 223 and 265 nm indicating the presence of an indolenine chromophore. The IR spectrum ($CHCl_3$) showed the absence of carbonyl functionalities.

High resolution mass spectral measurements afforded the molecular ion at m/z 556.3566, leading to the formula $C_{38}H_{44}N_4$, indicating the presence of 19 degrees of unsaturation in the molecule. Other important fragments appeared at m/z 525, 281, 208 and 124.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) was found to be fairly complex. A three-proton triplet at δ 0.65 ($J_{18,19}$ = 6.9 Hz) was assigned to methyl protons and a quartlet at δ1.70 ($J_{19,18}$ = 6.9 Hz) was assigned to the methylene protons of the ethyl side chain. The aromatic protons resonated in the region between δ7.2-7.6 as complex multiplets. The methylene and methine protons of bisstrictidine resonated as complex overlapping multiplets in the region between δ1.7-3.9. The signals for the ethyl group attached to a saturated carbon appeared at δ0.40 and δ0.96 for the methyl and

1

4

2

5

3

6

the methylene protons respectively. The downfield signals at $\delta 0.65$ and $\delta 1.72$ assigned to methyl and methylene protons of ethyl group and suggested a double bond between C-20 and C-21 and both C-20 and C-21 are quaternary carbons.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) showed several interesting features. Out of 38 carbons only 19 carbon signals were observed in the spectrum. This indicated that the alkaloid has a symmetrical dimeric structure. A downfield signal at $\delta 192.20$ was assigned to the C-2, α-to the indolenine nitrogen. A signal at $\delta 78.70$ was assigned to the C-16 methine carbon atom. Two downfield signals were observed for the carbons α-to the N_b nitrogen, resonating at $\delta 51.40$ and $\delta 55.12$ which were assigned to C-3 and C-5 methylene carbons respectively. The ^{13}C-NMR shift assignments are presented around structure 3.

On the basis of above spectral data, structure 3 was assigned to the alkaloid, named "bisstrictidine".

Didemethoxycarbonyltetrahydrosecamine (DDCTHS) (4)[16]

The UV spectrum (MeOH) of this new alkaloid was characteristic of the indole chromophore with maxima at 224, 284 and 290 nm. The IR spectrum ($CHCl_3$) exhibited bands at 3500 cm^{-1} (N-H), 2880 cm^{-1} (C-H), but did not show peaks in the carbonyl region.

The high resolution mass spectrum showed a weak molecular ion peak at m/z 564. The most prominent feature of the mass spectrum was a very strong base peak at m/z 126, characteristic of the tetrahydrosecamine system containing a saturated 3-ethyl piperidine ring. Accurate mass measurements of the M^+ peak could not be achieved with an EI source. However, a strong MH^+ signal could be recorded using FAB mass and accurate mass measurements using KI as an internal standard afforded the exact mass to be 564.4141 ($C_{38}H_{52}N_4$).This showed the presence of 15 double bond equivalents in the molecule. The mass spectrum

indicated that postsecamidine contained two indole units to which two C_9H_8N units are attached.

The ^1H-NMR spectrum ($CDCl_3$, 300 MHz) showed the presence of a six-proton triplet centred at $\delta 0.69$ ($J = 7.3$ Hz) which was assigned to the methyl protons and a four-proton multiplet centred at $\delta 1.16$ was assigned to the methylene protons of the ethyl group. The use of spin-spin decoupling as well as 2D-NMR (COSY-45) established the coupling between these.

The ^1H-NMR and mass spectroscopic data as well as biogenetic rational agrees with structure (4) assigned to DDCTHS. On the basis of these spectroscopic studies, structure 4 was proposed for didemethoxycarbonyltetrahydrosecamine.

17′-Hydroxyrhazisidine (5)[17]

This new alkaloid gave a pink colour reaction with ceric sulphate solution. Its UV spectrum showed absorptions at 222, 282 and 290 nm, characteristic for indoles. The high resolution mass spectrum afforded the molecular ion at m/z 632.3750 corresponding to the molecular formula $C_{40}H_{48}N_4O_3$ indicating nineteen degrees of unsaturstion in the molecule.

The ^1H-NMR spectrum ($CDCl_3$, 300 MHz) showed the presence of a six- proton triplet at $\delta 0.89$ ($J = 7.0$ Hz) for the C-18′ methyl protons. A four- proton distorted quartet occurring at $\delta 1.67$ were assigned to the C-19 and C-19′ protons. A three-proton singlet at $\delta 3.58$ indicated the presence of the ester methyl group. A one-proton downfield doublet at $\delta 5.34$ ($J_{15,14} = 5.5$ Hz) was assigned to the C-14 olefinic proton. Complex multiplets in the region between $\delta 7.10$-7.70 were assigned to the aromatic protons in the molecule. A one -proton singlet at $\delta 7.80$ was assigned to the N-H proton.

15-β-Hydroxyvincadifformine (6)[18]

The UV spectrum (MeOH) of this new compound showed absorptions at 205, 224, 296 and 327 nm, indicating the anilinoacrylate chromophore. The IR spectrum ($CHCl_3$) indicated the presence

of an ester carbonyl. The high resolution mass spectrum afforded the molecular ion peak at m/z 354.1943 corresponding to the formula $C_{21}H_{26}N_2O_3$, indicating eleven double bond equivalents in the molecule. The peak at m/z 323, ($C_{20}H_{23}N_2O_2$), corresponded to the loss of 31 m.u. (OCH_3) from the molecular ion. Other prominent peaks occurred at m/z 253, 222, 210, 180, 140 and 124. The fragment ions at m/z 210 and 124 are a common feature of *Aspidosperma* alkaloids.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) indicated the presence of ethyl side chain, since it showed a triplet at δ0.67 ($J_{18,19}$ = 7.5 Hz) for the C-18 methyl while the C-19 α and β protons appeared at δ0.96 and δ1.11, showing their non-equivalence. The C-15 proton resonated at δ3.74 as a multiplet, indicating the presence of an oxygen function at this carbon. The singlet at δ3.96 was assigned to the C-21 protons, its downfield shift indicating the α-stereochemistry for this proton. The ester methyl appeared at δ 3.74, while the indolic NH appeared at δ8.93. The ^{1}H-^{1}H couplings were established by carrying out two-dimensional NMR (COSY 45, 2D J-resolved) experiments.

Leepacine (7)[19]

This new alkaloid showed UV (MeOH) absorptions at 207, 250 and 298 nm, characteristic for the dihydroindole chromophore. The IR spectrum ($CHCl_3$) of the substance showed intense absorptions at 3350 cm^{-1} (N-H), 1735 cm^{-1} (ester C=0), 1720 cm^{-1} (ketone = C = 0), 1605 cm^{-1} (C=C) and 750cm^{-1} (aromatic C-H).

The high resolution mass spectrum of the alkaloid showed the molecular ion peak at m/z 350.1619, corresponding to the molecular formula $C_{21}H_{22}N_2O_3$, indicating twelve double bond equivalents in the molecule. The peak at m/z 322.1601 ($C_{20}H_{22}N_2O_2$) indicated the loss of 28 m.u. (M-CO) commonly observed in ajmaline type alkaloids. The peak at m/z 292.1575

($C_{19}H_{20}N_2O$) indicated the loss of ester group as 58 m.u., while the peak at m/z 263 suggested the loss of CHO from m/z 292.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) of leepacine showed a three-proton doublet at δ1.59 for the methyl group of the ethylidine side chain showing vicinal coupling with an adjacent olefinic proton ($J_{18,19}$ = 6.9 Hz). The C-19 olefinic proton on the other hand resonated as a quartet at δ5.70 showing vicinal coupling with the C-18 methyl protons ($J_{19,18}$ = 6.9 Hz). The C-3 proton resonated as a doublet of double doublets at δ3.24 ($J_{3,14}$ = 10.0Hz, $J_{3,14}\sim$1Hz, $J_{3,2}$ < 1Hz), its upfield chemical shift suggesting α stereochemistry. The low value of the coupling constants of the C-3 proton with the C-14 proton as well as with the C-2 proton also suggested α-configuration of the proton at C-3. A broad singlet at δ3.95 was assigned to the C-2 proton. This must be in β-configuration since the band with of the signal was found to be characteristically low (< 1Hz). The low value of this coupling constant is an account of the dihederal angle between C-2H and C-3H being close to 90°. The ^{13}C-NMR assignments are presented around structure 7.

Two dimensional NMR (2D J-resolved, COSY-45), NOE difference measurements and ^{13}C-NMR experiments were carried out to verify the assignments. The NOE interactions between the C-15 and C-18 protons, indicated that the 19,20-double bond is in an 'E'-configuration. The NOE difference measurements also served to establish the β and α stereochemistry for the C-2 and C-3 protons respectively.

N_b-Methyl Strictamine (8)[20]

The UV spectrum (MeOH) of this new alkaloid exhibited a characteristic indolenine absorptions at 222 and 265 nm. The IR spectrum ($CHCl_3$) showed an strong absorption at 1740 cm^{-1} indicating the presence of an ester group in the molecule.

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The high resolution mass spectrum afforded the molecular ion at m/z 337.1932 corresponding to the molecular formula $C_{21}H_{25}N_2O_2$, representing eleven degrees of unsaturation in the molecule. Other important fragments were present at m/z 322, 308 and 263. The mass fragmentation pattern was found to be very similar to that of strictamine[21].

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed a three proton doublet at δ1.56 ($J_{18,19}$ = 6.9 Hz) for the ethylidine methyl protons. The C-19 olefinic proton appeared at δ5.89 ($J_{19,18}$ = 6.9 Hz) as a quartet. The downfield chemical shifts for the C-3, C-5 and C-21 protons are due to the N^- function α-to them. The ester methyl protons resonated at δ3.68 as a singlet while the N^+-methyl protons appeared at δ3.80. The presence of four proton signals in the aromatic region suggested the presence of an unsubstituted indolenine nucleus.

The ^{1}H-^{1}H coupling was confirmed by the COSY-45 spectrum while the multiplicities of proton signal were determined by 2D J-resolved spectrum. NOE difference measurements were carried out to confirm the stereochemistry at the asymmetric centres which established 'E' configuration of 19,20 double bond and 'R' configuration at C-16. The ^{13}C-NMR assignments are presented around structure 8.

Rhazigine (9)[22]

This new alkaloid showed UV (MeOH) absorptions at 225, 282, 290 and 329 nm. The IR ($CHCl_3$) spectrum indicated the presence of an ester group by absorption at 1730 cm^{-1}.

High resolution mass spectral studies afforded the molecular ion peak at m/z 618.3987 corresponding to the molecular formula $C_{40}H_{50}N_4O_2$, indicating 18 double bond equivalents in the molecule. Other prominent peaks appeared at m/z 617, 616, 588, 335, 251 and 124.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed complex overlapping signals. A three-proton triplet at δ0.90 ($J_{18',19'}$ = 6.2 Hz) was assigned to the methyl protons of ethyl group. A multiplet at δ1.88 was assigned to the C-19 methylene protons of the ethyl side chain. A three proton triplet at δ0.66 ($J_{18,'19'}$ = 7.4 Hz) was assigned to the C-18′ protons of the ethyl group. A two proton multiplet at δ2.02 was assigned to the C-19′ methylene protons. The ester methyl protons resonated at δ3.68. A downfield signal at δ 7.80 was assigned to the indolic N-H proton. The signal for the C-12 proton was observed at δ7.46 as a doublet ($J_{12',12'}$ = 7.3 Hz). Other aromatic protons resonated as complex multiplets in region between δ6.90 – δ7.30. A one proton distorted doublet observed at δ5.34 (J = 5.3 Hz) was assigned to the C-15′ olefinic proton while another distorted doublet resonating at δ5.59 (J = 8.0 Hz) was assigned to the C-15 olefinic proton.

On the basis of above spectroscopic studies the structure 9 was assigned to rhazigine.

Rhazimine (10)[23,24]

This new alkaloid showed UV (MeOH) absorptions at 222, 265 and 290 nm. The IR spectrum showed absorptions at 1740 cm^{-1} (keto C = O), 1720 cm^{-1} (ester C = O) and 1630 cm^{-1} (C = C).

High resolution mass spectrum afforded the molecular ion peak at m/z 350.1619, corresponded to the molecular formula $C_{21}H_{22}N_2O_3$ indicating the presence of twelve double bond equivalents in the molecule. Other fragments were present at m/z 322.1668 ($C_{20}H_{22}N_2O_2$), 214.0864 ($C_{13}H_{12}NO_2$), 182.0603 ($C_{12}H_8NO$), 167.0738 ($C_{12}H_9N$) and 122.0967 ($C_8H_{12}N$).

The ^{1}H-NMR ($CDCl_3$, 300 MHz) spectrum showed a three proton double doublets at δ1.58 was assigned to the ethylidine methyl group showing vicinal coupling ($J_{18,19}$ = 7.0 Hz) with the adjacent C-19 olefinic proton and homoallylic coupling ($J_{18,21}$ =

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2.2 Hz) with the C-21 protons. The C-19 olefinic proton appeared at $\delta 5.57$ ($J_{19,18}$ = 7.0 Hz) showing vicinal coupling with the C-18 methyl protons. A three proton singlet at $\delta 3.51$ was assigned to the ester methyl. A low field singlet at $\delta 7.70$ was of particular significance, its position being in agreement with that expected for an olefinic proton attached to a ketimine carbon.

On the basis of these data structure 10 was originally proposed for rhazimine[23], but in the light of X-ray studies[24] the rhazimine was shown to have structure 10A. The ^{13}C-NMR assignments are presented around structure 10A.

Strictamine N-oxide (11)[25]

The UV spectrum (MeOH) of this alkaloid showed absorptions at 213 and 262 nm, indicating the indolenine chromophore. The IR spectrum ($CHCl_3$) showed the presence of an ester carbonyl group at 1740 cm^{-1}.

The high resolution mass spectrum afforded the molecular ion peak at m/z 338.1625, which corresponded to the formula $C_{20}H_{22}N_2O_3$, indicating eleven double bond equivalents in the molecule. The mass fragmentation pattern of alkaloid is very similar to that reported for strictamine[21].

The ^{1}H-NMR ($CDCl_3$, 300 MHz) spectrum showed a three proton doublet at $\delta 1.62$ ($J_{18,19}$ = 7.0 Hz, $J_{18,21}$ = 2.5 Hz) assigned to the methyl of an ethylidine group showing vicinal coupling with the adjacent C-19 proton and homoallylic couplings with the C-21 protons. A one proton quartet at $\delta 5.75$ ($J_{19,18}$ = 7.0 Hz) was assigned to the C-19 olefinic proton showing vicinal coupling with the C-18 methyl protons. A doublet at $\delta 2.15$ ($J_{16,15}$ = 3.2 Hz) was assigned to the C-16 proton, the upfield shift being on account of the shielding influence of the indolenine nucleus on which it overlies. A characteristic one-proton doublet for the C-3 proton appeared at the rather-downfield position at $\delta 5.61$ ($J_{3,14}$ = 6.2 Hz)

on account of the deshielding influence of the vicinal quaternary nitrogen. The ester methyl protons appeared at δ3.73.

Because of the strong similarities of the ^{1}H-NMR and the mass spectrum with those of strictamine[21], as well as the polar nature of the compound it was suspected that the substance isolated was strictamine-N-oxide. This was confirmed by deoxygenation in dichloromethane with PCl_3 to strictamine, and by comparison of its spectral data with that reported in the literature.

Strictanine (12)[26]

This new alkaloid showed UV spectrum (MeOH) characteristic for a dihydroindole chromophore with maxima at 212, 253, 280 and 290 nm. The IR spectrum ($CHCl_3$) showed intense absorptions at 3450 cm^{-1} (O-H), 2900 cm $^{-1}$ (C-H), 1680 cm^{-1} (C = O), 1590 cm^{-1} (C = C) and 750 cm^{-1} (aromatic CH).

The high resolution mass spectrum showed the molecular ion peak at m/z 326.1975 leading to the molecular formula $C_{20}H_{26}N_2O_2$, indicating nine double bond equivalents in the molecule. A fragment which occurred at m/z 309.1685 ($C_{20}H_{25}N_2O$) corresponded to the loss of hydroxyl gorup. The peak at m/z 297 indicated the loss of two different fragments, (i) m/z 297.1954 ($C_{19}H_{25}N_2O$) M^+-CHO and (ii) m/z 297.1647 ($C_{18}H_{21}N_2O_2$) M^+-C_2H_5. This clearly indicated the presence of an aldehydic group as well as an ethyl group.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) clearly indicated the presence of N-formyl group: the doublet for C-12H was shifted significantly downfield to δ8.03 in comparison to other aromatic protons. This was indicative of the presence of a carbonyl group at the nitrogen of the dihydroindole nucleus. The presence of four proton signals in the aromatic region indicated a non-substituted benzene ring. The aldehydic proton appeared at δ8.50, its upfield chemical shift suggestive of an N-formyl function. A poorly

resolved doublet at δ4.05 (J = 7.0 Hz) may be attributed to the C-2 proton, indicating the substitution of hydroxyl group at C-16. The singlet appearing at δ2.46 was assigned to the C-21H in β-stereochemistry, as earlier been observed in other closely related *Aspidosperma* alkaloids. On the basis of the above spectroscopic evidences the structure (12) was assigned to strictanine.

Strictanol (13)[14,26]

This new alkaloid showed UV (MeOH) absorptions at 227 and 290 nm, indicating the β-hydroxy indoline chromophore. Its IR (KBr) spectrum showed intense absorption at 2970-2860 cm^{-1} (C-H) and 758 cm^{-1} (aromatic C-H).

The high resolution mass spectrum afforded M^+ at m/z 298.2031, leading to the molecular formula, $C_{19}H_{26}N_2O$, indicating eight double bond equivalents in the molecule. The base peak occurred at m/z 281.2021 ($C_{19}H_{25}N_2$), indicating the loss of hydroxyl group. The presence of a prominent fragment at m/z 269.1712 ($C_{21}H_{21}N_2O$), resulting from the loss of the ethyl group from the molecular ion is a common feature of *Aspidosperma* alkaloids.

The ^1H -NMR spectrum (CD_3OD, 300 MHz) indicated the presence of 26 protons. The methyl protons of the ethyl side chain appeared as a triplet at δ0.93 ($J_{18,19}$ = 7.6 Hz). The adjacent methylenic protons (C-19H) resonated as a quartlet at δ1.38 ($J_{19,18}$ = 7.6 Hz). The C-21 α proton appeared at δ3.13 as a doublet ($J_{21\alpha, 21\beta}$ = 12.2 Hz), while the C-21 β proton resonated at δ3.62 ($J_{21\beta, 21\alpha}$ = 12.2 Hz). The C-3α proton appeared at δ3.39 as a doublet ($J_{3\alpha,14}$ = 8.1 Hz) while another doublet at δ3.71 ($J_{3\beta,14}$ = 8.1 Hz) was assigned to the C-3β proton. The downfield chemical shift of C-3 and C-21 protons are on account of the adjacent electron-withdrawing nitrogen function. The ^{13}C-NMR assignments are presented around structure 13.

The structure and stereochemistry of strictanol (13) has been investigated by extensive NMR studies (2D J-resolved, COSY-45, NOESY), ^{13}C-NMR, hetero-COSY experiments and NOE difference measurements.

Stricticine (14)[27]

The UV spectrum (MeOH) of this alkaloid gave absorptions at 208, 228, 292 and 327 nm, characteristic of the anilinoacrylate chromophore. The IR spectrum ($CHCl_3$) showed absorptions at 3400 cm^{-1} (N-H) and 1690 cm^{-1} (ester carbonyl).

The high resolution mass spectrum afforded M^+ at m/z 338.1615 in agreement with the formula $C_{20}H_{22}N_2O_3$, indicated eleven double bond equivalents in the molecule. Other prominent peaks occurred at m/z 293, 269, 254, 235, 223, 208, 194 and 180.

The ^1H-NMR spectrum ($CDCl_3$, 300 MHz) indicated the presence of twenty-two protons. The coupling interactions between coupled protons were confirmed by spin decoupling experiments and from the COSY-45 spectrum. An upfield three-proton doublet at δ0.99 ($J_{18,19}$ = 5.5 Hz) was assigned to the C-18 methyl protons, while a one proton multiplet at δ2.83 was assigned to the C-19 proton. A one proton broad singlet at δ3.62 was assigned to the C-3 proton, its chemical shift suggesting β-stereochemistry. The indole N-H appeared at δ8.76. The ^{13}C-NMR assignments are presented on structure 14.

The stereochemistry at the various asymmetric centre was established by carrying out NOE difference studies. This established that the epoxide bearing carbons C-19 and C-20 possessed 'S' configuration. These NOE results also established that the C-18 methyl group overlying the cyclohexane ring system possesses a *cis* configuration.

Strictimidine (15)[28]

The UV spectrum (MeOH) of this new compound showed absorption at 210, 222 and 263, indicating the indolenine chromophore. The IR spectrum ($CHCl_3$) showed no absorption peaks in the carbonyl region but showed intense peaks at 3400 cm-1 (O-H), 2900 cm^{-1} (C-H), 1605 cm^{-1} (C = C).

The high resolution mass spectrum indicated the molecular ion peak at m/z 296.1888, corresponding to the molecular formula $C_{19}H_{24}N_2O$, indicating nine double bond equivalents in the molecule. The peak at m/z 279.1602 ($C_{19}H_{23}N_2$) corresponded to the loss of hydroxyl group.

In the ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) the C-18 methyl protons appeared at δ 0.38 as a triplet ($J_{18,19}$ = 7.6 Hz); the C-19α and β protons resonated at δ0.87 and δ1.12 indicating their non-equivalence on account of their prochiral nature. The C-21 proton appeared at δ2.60 as a broad singlet, its downfield chemical shift suggesting α-stereochemistry. A doublet at δ3.66 ($J_{15,14}$ = 6.7 Hz) was assigned to the C-15 proton having α-stereochemistry, its downfield chemical shift indicating the presence of oxygen function at this carbon.

Strictimine (16)[29]

The UV spectrum (MeOH) of this new alkaloid showed the lack of any chromophoric grouping in the molecule. The IR ($CHCl_3$) showed intense absorptions at 2850 cm^{-1} (C-H) and 1710 cm^{-1} (C = O).

The high resolution mass spectrum afforded the molecular ion at m/z 252.2195, corresponding to the molecular formula $C_{15}H_{28}N_2O$, indicating the presence of three double bond equivalents in the molecule. The mass spectrum indicated that the substance was composed of two ethyl piperidine units. The molecular ion at m/z 252.2195 lost one ethyl group to afford the

peak at m/z 233.1805 ($C_{13}H_{23}N_2O$). Alternatively it lost one of the ethyl piperidine units to afford the ion at m/z 140.1080 ($C_8H_{14}NO$) which contained the remaining ethyl piperidine unit and a carbonyl group. The facile loss of C=O from the fragment at m/z 140.1080 ($C_8H_{14}NO$) to afford the ions at m/z 112.1128 ($C_7H_{14}N$) indicated that the carbonyl group was not a part of the piperidine ring but was bonded externally to one of the ring carbon atoms or to the nitrogen. Attempted reduction with sodium borohydride failed to afford the corresponding alcohol indicating that the carbonyl group was not present as a ketone.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed the presence of a 6-H distorted triplet at δ0.89 (J = 6.5 Hz) which was assigned to the methyl protons of the two ethyl groups. Two downfield multiplets at δ3.52 and δ3.65 were assigned to the C-2α and β protons. Spin-spin decoupling experiments established that the C-3 and C-3′ methine protons were located as multiplets at δ 2.35. The ^{13}C-NMR assignments are shown on structure 16.

Strictine (17)[30]

The UV spectrum (MeOH) of this new alkaloid showed absorptions at 222 and 295 nm, characteristic for the indole chromophore. The IR spectrum ($CHCl_3$) showed intense absorptions at 1738 cm^{-1} (ketonic C=O), 1670 cm-1 (ester C=O) and 1620 cm^{-1} (C=C).

The high resolution mass measurements afforded the molecular ion peak at m/z 336.1462 leading to the molecular formula $C_{20}H_{20}N_2O_3$, indicating twelve double bond equivalents in the molecule.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed the presence of 20 protons, each of which was identified by a series of homodecoupling experiments and further substantiated by recording COSY-45 spectrum. A three proton singlet at δ2.29 was

assigned to the methyl protons of the acetyl group. Another three proton singlet at δ3.57 was consistent with the presence of methyl protons of the carbomethoxy group. A downfield doublet at δ4.60 ($J_{16,15}$ = 4.5 Hz) was assigned to C-16H, which is characteristic for compounds bearing a mavacurine type skeleton[31]. A rather downfield one proton broad singlet at δ7.10 was assigned to the olefinic proton at C-21, its lowfield value being consistent with the presence of an adjacent nitrogen function. The C-3 proton resonated at δ 3.60 ($J_{3,14}$ = 6.0 Hz) as a doublet, its chemical shift suggesting α-stereochemistry at C-3. The ^{13}C-NMR assignments are presented on structure 17.

Strictalamine (18)[32]

The UV spectrum (EtOH) of this new alkaloid showed absorptions at 218 and 265 nm, characteristic of the indolenine chromophore. The IR specturm ($CDCl_3$) showed the presence of formyl group at 1720 cm^{-1}.

The mass spectrum showed the molecular ion peak at m/z 292.1572 corresponding to the molecular formula $C_{19}H_{20}N_2O$ indicating eleven double bond equivalents in the molecule. Other important peaks appeared at m/z 234, 263 and 264. The peak at m/z 263 suggested the loss of formyl group from the molecular ion. The mass fragmentation pattern was found to be identical with that of strictamine[21].

The ^{1}H-NMR spectrum ($CDCl_3$, 60 MHz) showed the presence of an ethylidine side chain. The methyl of the ethylidine group appeared at δ1.60 as a doublet ($J_{18,19}$ = 7.0 Hz) while the C-19 olefinic proton resonated at δ5.4 as a multiplet. A doublet at δ4.80 was assigned to the C-16 proton. The downfield chemical shift was ascribed to the deshielding effect of the indolenine nucleus. The formyl proton resonated at δ8.75 as a singlet. The aromatic protons appeared at δ7.10-7.80 multiplets. The presence of four protons in

the aromatic region suggested the lack of substitution in the indolenine nucleus.

On the basis of the above spectroscopic data structure 18 was proposed for strictalamine. This was also established by reduction of strictamine[21] with lithium aluminium hydride to the alcohol which on oxidation gave a compound identical with strictalamine. This established the structure 18 for strictalamine.

(B) NEW INDOLE ALKALOIDS FROM CATHARANTHUS ROSEUS

Catharanthus roseus L.G. Don (*Apocynaceae*) is one of the most thoroughly investigated plants and it owes its reputation to the presence in it of vinblastine and vincristine, two powerful anticancer alkaloids from its leaves, which find wide use in medicine[33-38]. The work on the alcoholic extract of the leaves has resulted in the isolation and structure elucidation of the following new alkaloids.

Alioline (19)[39]

A novel alkaloid was isolated from the leaves of *Catharanthus roseus*. Its UV spectrum (MeOH) was typical of indoles with absorptions at 227, 283 and 292 nm. The IR spectrum ($CHCl_3$) showed intense absorptions at 3130 cm^{-1} (N-H), 2910 cm^{-1} (C-H), 1720 cm^{-1} (ester C=O), 1680 cm^{-1} (ketonic C=O), 1600 cm^{-1} (C=C) and 750 cm^{-1} (aromatic C-H).

The HRMS showed the molecular ion at m/z 472.2720 consistent with the molecular formula $C_{30}H_{36}N_2O_3$, indicating fourteen double bond equivalents in the molecule. The peak at m/z 335.1773 ($C_{21}H_{23}N_2O_2$) corresponding to the loss of 137 m.u. ($C_9H_{13}O$) from the molecular ion, established the substitution at C-15. The molecule was seen to be cleaved to afford a peak at m/z 271.1916 ($C_{18}H_{25}NO$) containing the five-membered ring moiety, while the ion at m/z 201.0723 ($C_{12}H_{11}NO_2$) comprised the indolic portion of the molecule.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed a three-proton triplet at δ0.27 ($J_{18,19}$ = 7.4 Hz). Its rather upfield chemical shift is attributed to its falling in the shielding zone of olefinic bond in the five membered ring. A broad singlet at δ2.58 was assigned to the C-21 proton, its upfield chemical shift suggesting α-stereochemistry. A three-proton singlet at δ3.78 was assigned to the ester methyl protons while two other singlets at δ1.45 and δ1.80 were assigned to the C-4′ methyl and the acetyl methyl on the five-membered ring respectively. The C-1′ methyl appeared at δ0.66 as a singlet, its upfield chemical shift being consistent with the shielding influence of carbonyl function at C-3′. A one-proton singlet at δ 5.37 was assigned to the C-5′ proton. The presence of four protons in the aromatic region suggested the lack of substitution of the indole chromophore. The indolic NH resonated as a singlet at δ 8.55.

The spin-spin coupling interactions were established through the COSY-45 spectrum while the multiplicity of the overlapping proton signals were determined from the 2D J-resolved spectrum. The NOESY spectrum served to establish spatial proximities while NOE difference measurements confirmed the relative stereochemistry at various asymmetric centres. The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) showed the presence of thirty carbon atoms. The multiplicity assignments were made by DEPT experiments, and the assignments confirmed by carrying out hetero-COSY experiments. On the basis of above data, structure 19 was proposed for alioline. The ^{13}C-NMR assignments are presented on structure 19.

Bannucine (20)[40]

The UV spectrum (MeOH) of this new alkaloid showed absorptions at 238 and 280 nm, showing a slight bathochromic shift from the normal dihydroindole chromophore. The IR spectrum

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($CHCl_3$) showed absorptions at 3400 (N-H), 3200 (O-H), 1710 (ester C=O) and 1690 cm^{-1} (amidic C=O).

The HRMS showed molecular ion peak at m/z 539.2612 which was consistent with the formula $C_{29}H_{37}N_3O_7$, indicating thirteen double bond equivalents in the molecule, differing from vindoline by 83 m.u. (C_4H_5NO). The fragment at m/z 84.0449, suggested the presence of C_4H_6NO unit in the molecule. Linked scan measurements were also carried out to determine the fragmentation pathway.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) of bannucine showed two 3H singlets at δ3.83 and δ2.07 which were assigned to the methoxy carbonyl and acetoxy methyl groups respectively. The OCH_3 group on the aromatic ring resonated as a 3H singlet at δ 3.79, while the N-CH_3 protons appeared as another 3H singlet at δ2.65. The methyl protons of ethyl side chain appeared as a triplet at δ 0.44 ($J_{18,19}$ = 7.3 Hz). A doublet at δ5.20 ($J_{15,14}$ = 10.3 Hz) was assigned to the C-15 olefinic proton while the C-14 olefinic proton resonated at δ5.82. Examination of the aromatic region of bannucine showed that only two aromatic protons were present at δ6.08 and δ6.90 each resonating as a sharp singlet. The position of these resonances as well as the lack of *ortho* and *meta* coupling agreed with their being assigned to the C-9 and C-12 protons respectively, indicating that the lactam substituent was attached to C-10. The protons of the five-membered lactam ring substituent at C-10 were readily recognized in the ^{1}H-NMR spectrum.

Two-dimensional NMR measurements (COSY-45, 2D J-resolved, NOESY) fully agreed with the proposed structure 20 for bannucine. The NOESY spectrum established the relative stereochemistry of several key functionalities in bannucine.

The ^{13}C-NMR spectrum is also consistent with the proposed structure 20. The ^{13}C-NMR shift assignments are presented around structure 20.

In view of the above data, structure 20 was assigned to bannucine. It is the first *Aspidosperma* alkaloid bearing a five-membered lactam substituent.

Fluorocarpamine-N-oxide (21)[41]

The UV spectrum (MeOH) of this new alkaloid showed absorptions at 235, 257 and 298 nm, characteristic of dihydroindole chromophore. The IR spectrum ($CHCl_3$) afforded absorptions at 1740 cm^{-1} (ester C=O), and 1685 cm^{-1} (ketonic C=O).

The HRMS afforded molecular ion peak at m/z 354, with characteristic loss of oxygen as encountered in N-oxides. Other major peaks were present at 338, 279, 265, 231, 193, 160 and 121. The fragmentation pattern was remarkably similar to that of fluorocarpamine[42].

The ^{1}H-NMR spectrum ($CDCl_3$, 100 MHz) was similar to that of fluorocarpamine[42]. The substance was treated with PCl_3 in $CHCl_3$. The product was compared with an authentic sample of fluorocarpamine. The polar nature of the material, its characteristic mass spectrum and its readily deoxygenation with PCl_3 to fluorocarpamine unambiguously established it to be fluorocarpamine-N-oxide (21).

Gomaline (22)[43]

The UV spectrum (MeOH) of gomaline showed absorptions at 210 and 262 nm, characteristic of the indolenine chromophore. The IR spectrum ($CHCl_3$) showed absorptions at 1725 cm^{-1} (ester C=O) and 3400 cm^{-1} (O-H).

The HRMS bore a distinct resemblance to that of strictamine[21] showing the molecular ion peak at m/z 338.1618, consistent with the molecular formula $C_{20}H_{22}N_2O_3$, indicating eleven double bond equivalents in the molecule. Other major peaks appeared at m/z 321, 279, 261, 232, 206, 180 and 115.

The ^{1}H-NMR spectrum ($CDCl_3$, 100 MHz) showed a one-proton triplet at $\delta 5.57$ ($J_{19,18}$ = 7.0 Hz) for an olefinic proton while a two-proton doublet at $\delta 3.94$ ($J_{18,19}$ = 7.0 Hz) was assigned to the hydroxymethylene protons in an α-disposition. A three-proton singlet at $\delta 3.79$ was assigned to the ester methyl group. A doublet at $\delta 4.68$ ($J_{3,14}$ = 4.8 Hz) was assigned to the C-3 proton.

The stereochemistry at C-16 emerges from the fact that in the opposite configuration at this centre the proximity with the ketimine group of indolenine system causes an upfield shift of the ester methyl[44]. The stereochemical disposition at C-19 cannot be defined with any certainty, but in view of our earlier establishment of the structure and absolute configuration of the picraline group of bases[45] structure 22 was tentatively proposed for gomaline.

Leurosinone (23)[46]

The UV spectrum (MeOH) of compound 23 showed absorptions at 214, 260 and 296 nm, indicating the presence of both indole and dihydroindole chromophores. The IR spectrum ($CHCl_3$) showed absorptions at 3460 (N-H and O-H) and 1730 cm^{-1} (ester C=O). Interestingly an additional carbonyl absorption was observed at 1710 cm^{-1}.

The HRMS showed the molecular ion at m/z 864.4349, corresponding to the molecular formula $C_{49}H_{60}N_4O_{10}$, indicating twenty-two degree of unsaturation in the molecule. The overall fragmentation pattern was very similar to that of leurosine[47]. The peak at m/z 208.1327 ($C_{12}H_{18}NO_2$) indicated that the -CH_2COCH_3 unit was attached in the vicinity of the piperidine unit. The possibility of the -CH_2COCH_3 group being present in the vindoline half of the molecule was eliminated from the fact that the normal fragmentation of the vindoline was observed[47].

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed that a vindoline moiety substituted at the 10-position was present. Two

3H singlets at δ3.78 and δ2.12 were assigned to the methyl groups of the 16-carbomethoxy and 17-acetoxy groups respectively. The 11-OCH_3 group on the aromatic ring resonated as a 3H singlet at δ 3.80 while the N-CH_3 protons appeared as another 3H singlet at δ 2.70. The ^{1}H-NMR spectrum showed the presence of ethyl side chain and epoxymethine protons. Two striking differences on comparison with the ^{1}H-NMR resonances of leurosinone with that of leurosine, were the presence of an additional 3H singlet at δ2.09 assigned to the methyl of an acetyl group and the absence of the doublet for the 5′β-proton at δ3.67[48], since the -CH_2COCH_3 group was attached at this position in a β-configuration in leurosinone 23.

A series of NOE difference measurements were carried out to ascertain the position and stereochemistry of the -CH_2COCH_3 group. The ^{13}C-NMR (GASPE and DEPT) experiments supported the structure 23 for leurosinone. The ^{13}C-NMR chemical shift assignments are presented around structure 23.

Rosamine (24)[49]

The UV spectrum (MeOH) of rosamine showed absorptions at 246, 280 and 340 nm, characteristic of the pseudoindoxyl system. The IR spectrum ($CHCl_3$) showed absorptions at 1720 cm^{-1} (ester C=O) and 1690 cm^{-1} (conjugated C=O).

The HRMS showed molecular ion peak at 352.1779, consistent with the formula $C_{21}H_{24}N_2O_3$, indicating eleven double bond equivalents in the molecule. Other major peaks were present at m/z 335, 293, 267, 216, 158, 135 and 107. The peak at m/z 158.0600 ($C_{10}H_8NO$) further suggested the presence of a pseudoindoxyl system.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) of rosamine showed a distinct resemblance to the ^{1}H-NMR spectrum of catharanthine[50]. A three-proton triplet appeared at δ1.0 ($J_{18,19}$ = 7.3 Hz) was assigned to methyl group of the ethyl side chain. The

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ester methyl group resonated at δ3.19, the upfield chemical shift being attributed to the shielding influence of the pseudoindoxyl system.

Rosicine (25)[51]

The UV spectrum (MeOH) of rosicine exhibited absorptions at 203, 223, 295 and 325 nm, characteristic of an analinoacrylate chromophore. The IR spectrum ($CHCl_3$) showed a strong absorption at 1670 cm^{-1} indicating the presence of an amide or a conjugated ester group.

The HRMS afforded the molecular ion peak at 324.1467, consistent with the formula $C_{19}H_{20}N_2O_3$ indicating the presence of eleven double bond equivalents in the molecule. The mass spectrum showed intense peaks at m/z 214 and 110 often encountered in Aspidosperma-type alkaloids bearing an anilinoacrylate skeletal system[52,53].

The ^{1}H-NMR spectrum ($CDCl_3$, 250 MHz) of rosicine was undertaken and the assignments were confirmed by the two dimensional COSY spectrum. A three-proton singlet at δ3.78 was assigned to the ester methyl group. The absence of any other methyl signal indicated that the ethyl or substituted ethyl side chain was absent in rosicine. A low field double-doublet at δ3.38 is assigned to the C-14 proton ($J_{14,3}$ = 5.2 Hz, $J_{14,15}$ = 3.8 Hz). Another lowfield one-proton doublet at δ3.17 is assigned to the C-15 proton. ($J_{15,14}$ = 3.8 Hz). A multiplet at 1.89 was assigned to the C-20 proton. The aromatic protons resonated as complex multiplets in the range of δ6.84-7.28.

The ^{13}C-NMR (GASPE) spectrum supported the structure 25 for rosicine. The ^{13}C-NMR shift assignments are presented in structure 25.

(C) NEW INDOLE ALKALOIDS FROM ALSTONIA MACROPHYLLA

Alstonia macrophylla Wall is a common plant in Sri Lanka. Several studies on this species growing in other countries have been reported[54-59]. The plant has found wide use in medicinal preparations in the Philippines[60]. The ethanolc extract of the leaves of *A. macrophylla* of Sri Lankan origin obtained under a joint programme with Sri Lankan scientists afforded the following new alkaloids.

Alstonamide (26)[61]

The UV spectrum (MeOH) of alstonamide showed absorptions at 208, 264 and 300 nm, characteristic for dihydroindole nucleus. The IR spectrum ($CHCl_3$) showed intense absorptions at 2920 cm^{-1} (C-H), 1723 cm^{-1} (ester C=O), 1657 cm^{-1} (N-formyl C=O) and 1600 cm^{-1} (C=C).

The HRMS afforded M^+ at m/z 412.1987, consistent with the molecular formula $C_{23}H_{28}N_2O_5$ indicating eleven degrees of unsaturation in the molecule. The peaks at m/z 383 and 381 suggested the loss of CHO and OCH_3 groups while the peak at m/z 325 corresponded to the loss of ester group from m/z 384.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed a 3H double doublet at δ 1.60 ($J_{18,19}$ = 7.0 Hz, $J_{18,15} \sim J_{18,21}$ = 1.8 Hz) for the C-18 methyl. The C-19 olefinic proton resonated at δ5.43 as a split quartet ($J_{19,18}$ = 7.0 Hz, $J_{19,15} \sim J_{19,21} \sim$ 1 Hz). A broad singlet at δ3.72 was assigned to the C-2 proton in its β-configuration. The ester methyl protons resonated at δ3.86 as a singlet while the other 3H singlets at δ3.81 and δ3.82 were asssigned to the 10-OCH_3 and 11-OCH_3 protons respectively. Two singlets at δ7.79 and δ7.05 were assigned to the C-9 and C-12 protons. The downfield chemical shifts for the C -9H and C-12H were attributed to the deshielding effect caused by the N-formyl function. The presence of two proton

signals in the aromatic region indicated the existence of a disubstituted indole nucleus. A one-proton singlet at δ8.47 was assigned to the N-formyl function.

Two dimensional NMR measurements (COSY-45°. 2D J-resolved) were carried out to verify the assignments. The NOE difference measurements were carried out to establish the stereochemistry at various asymmetric centres. It established 'E' stereochemistry of the ethylidine side chain.

On the basis of the above spectroscopic studies, structure 26 was assigned to alstonamide.

Alstopicralamine (27)[62]

The UV spectrum (MeOH) of this new alkaloid showed absorptions at 230, 245 and 300 nm, revealing the presence of a dihydroindole system. The IR spectrum ($CHCl_3$) displayed intense absorptions at 1723 cm^{-1} (ester C=O), 1600 cm^{-1} (C=C) and 1280 cm^{-1} (C-O).

The HRMS afforded molecular ion peak at m/z 412.1813 in agreement with the formula $C_{23}H_{28}N_2O_5$, indicating eleven double bond equivalents in the molecule. The peak at m/z 353 arose by the loss of carbomethoxy group from M^+. A peak at m/z 135 corresponded to the substituted piperidine ion. The fragmentation pattern were distinctly similar to that of other picraline bases.

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) showed a three-proton double doublet at δ1.51 ($J_{18,19}$ = 7.0 Hz, $J_{18,21}$ = 2.4 Hz) assigned to the methyl of the ethylidine side chain while the C-19 olefinic proton appeared at δ5.52 ($J_{19,18}$ = 7.0 Hz) as a quartet. A three-proton singlet at δ2.94 was due to the N-CH_3 proton. The spectrum showed three 3-H singlets at δ3.65, 3.75 and 3.86 corresponding to two methoxy groups and one carbomethoxy group. The ester methyl protons appeared at δ3.65 as a singlet. The

2D COSY-45 spectrum confirmed the ^1H-^1H couplings in the molecule.

On the basis of the above studies, structure 27 was assigned to alstopicralamine.

Alstozine N-oxide (28)[63]

The UV spectrum (MeOH) of this new alkaloid showed absorptions at 212, 245 and 307 nm, characteristic of a dihydroindole chromophore. The IR spectrum ($CHCl_3$) exhibited intense absorptions at 3660 cm^{-1} (O-H), 2900 cm^{-1} (C-H), 1724 cm^{-1} (ester C=O), 1595 cm^{-1} (C=C) and 980 cm^{-1} (C-O).

The HRMS displayed the molecular ion peak at m/z 400.1975 corresponding to the molecular formula $C_{22}H_{28}N_2O_5$, indicating the presence of ten double bond equivalents in the molecule. The peaks at m/z 384 and 383 suggested the loss of oxygen and hydroxyl group from the molecular ion. The overall mass fragmentation pattern was similar to those of Picralima alkaloids.

The ^1H-NMR spectrum ($CDCl_3$, 300 MHz) revealed a three-proton double doublet at δ1.47 ($J_{18,19}$ = 7.1 Hz, $J_{18,21}$ = 1.9 Hz) for the C-18 methyl protons. The vicinal vinylic proton appeared as a quartet at δ5.65 ($J_{19,18}$ = 7.1 Hz). A doublet at δ2.74 ($J_{16,15}$ = 4.0 Hz) was assigned to the C-16 bridghead proton. The C-3, C-5 and C-21 protons appeared downfield due to the N^+ function. A 3H singlet at δ2.63 was assigned to the N-CH_3 group while another 3H singlet at δ3.49 was due to the ester methyl protons. The aromatic region showed three one-proton signals suggesting a mono-substituted dihydroindole nucleus. A 3H singlet at δ3.68 was assigned to the aromatic methoxy protons.

Two dimensional NMR experiments (COSY-45°, 2D J-resolved) were carried out to verify the assignments. The NOE measurements suggested the 'E' stereochemistry of the ethylidine

side chain an α-stereochemistry of the hydroxyl group at C-2. The ^{13}C-NMR assignments are presented on structure 28.

N_b-Demethylalstophylline oxindole (29)[64]

The UV spectrum (MeOH) of this new alkaloid displayed characteristic absorptions for the oxindole system with absorptions at 223, 256, 286 and 294 nm. The IR spectrum ($CHCl_3$) showed absorptions at 1650 (conj. C=O) and 1705 cm^{-1} (lactam C=O).

The HRMS showed the molecular ion peak at m/z 368.1736, in agreement with the molecular formula $C_{21}H_{24}N_2O_4$, indicating eleven double bond equivalents in the molecule. The peak at m/z 179.0949 resulted from the cleavage of the spiran ring. The accompanying peaks at m/z 161 and 136 are associated with the loss of water and an acyl radical (CH_3CO) from m/z 179. The reto Diels-Alder type fragmentation of the ring D gave rise to the indole-containing fragment at m/z 244.

The ^1H NMR spectrum ($CDCl_3$, 300 MHz) showed three methyl singlets at δ2.24, 3.17 and 3.18. These signals were assigned to the acetyl methyl, N_a-methyl and methoxy groups respectively. The rather lowfield value of the N_a-methyl group suggested that the nitrogen bearing methyl group was adjacent to the lactam carbonyl group. The typical pattern of signals at δ6.45, 6.80 and 8.16 confirmed the presence of a methoxy substituent at C-11 of the aromatic nucleus. A low field singlet at δ7.62 was assigned to the C-21 olefinic proton, its downfield chemical shift value is because of it being in the β-position to the carbonyl group and the presence of an adjacent oxygen. The spin-spin coupling interactions were determined through the COSY-45 spectrum while the multiplicities of the proton signals were unambiguously determined by 2D J-resolved spectrum. In order to confirm the relative stereochemistry at various asymmetric centres NOE difference measurements were carried out.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) showed twenty-one carbon resonances. The multiplicity assignments were made by using DEPT experiments. These experiments revealed the presence of three methyl, three methylene and eight methine carbons in agreement with structure 29. The ^{13}C-NMR assignments are presented on structure 29.

On the basis of the above spectroscopic data, structure 29 was assigned to N_b-demethylastophylline oxindole.

16-Hydroxy-N_b-demethylalstophylline (30)[65]

The UV spectrum (MeOH) of 16-hydroxy-N_b-demethylalstophylline oxindole afforded absorptions at 218, 215, 235, 285 and 294 nm, characteristic of oxindole alkaloids. The IR spectrum ($CHCl_3$) showed absorptions at 1690 cm^{-1} (lactam C=O) and 1620 cm^{-1} (α,β-unsaturated C=O).

The HRMS showed the molecular ion peak at m/z 384.1672 corresponding to the formula $C_{21}H_{24}N_2O_5$ indicating the presence of eleven double bond equivalents in the molecule. The peak at m/z 354.1603 ($C_{20}H_{22}N_2O_4$) suggested the loss of CH_2O from the molecule. An important peak appearing at m/z 195.0895 ($C_{10}H_{13}NO_3$) after removal of a water molecule afforded the peak at m/z 177.0765 corresponding to the formula $C_{10}H_{11}NO_2$. This suggested that there is a hydroxyl gorup at C-16 in the molecule.

The ^1H-NMR spectrum ($CDCl_3$, 300 MHz) showed three methyl singlets at δ2.23, 3.14 and 3.82. These signals were assigned to the acetyl methyl, N_a-methyl and methoxy methyl protons, respectively. The rather lowfield value of N_a-methyl protons suggested that the nitrogen bearing methyl group was adjacent to the lactam carbonyl group. The typical pattern of signals at δ6.42, 6.77 and 8.05 confirmed the presence of a substituent (OMe) at C-11 of the aromatic nucleus. The NOE experiments served to establish the β-stereochemistry of proton at C-3 and C-5. It also

established the -α -stereochemistry of proton and hydroxyl group at C-11 and C-16 respectively.

On the basis of the above spectroscopic data, structure 30 was assigned to 16-hydroxy-N_b-demethylalstophylline.

19-Hydroxyvincamajine (31)[66]

The UV spectrum (MeOH) of this new alkaloid showed absorptions at 175 and 247 nm. The IR spectrum ($CHCl_3$) contained a carbonyl at 1730 cm^{-1}, and a band at 740 cm^{-1}, characteristic of an *ortho*-disubstituted benzene.

The HRMS showed the molecular ion peak at m/z 384.2036 corresponding to molecular formula $C_{22}H_{28}N_2O_4$, indicating twelve degrees of unsaturation in the molecule. The ^1H-NMR spectrum showed a close resemblance to that of vincamajine[67], except for the doublet at δ1.0 which was assigned to the C-19 methyl group.

On the basis of the above spectroscopic data, structure 31 was assigned to 19-hydroxyvincamajine.

Strictaminolamine (32)[68]

The UV spectrum (MeOH) of strictaminolamine was characteristic of the dihydroindole chromophore with absorptions at 207, 244 and 292 nm. The IR spectrum ($CHCl_3$) displayed strong absorptions at 1720 cm^{-1} (ester C=O) and 1595 cm^{-1} (C=C).

The HRMS showed the molecular ion peak at m/z 354.1947 corresponding to the molecular formula $C_{21}H_{26}N_2O_3$, indicating the presence of ten double bond equivalents in the molecule. The mass spectrum of the compound after deuterium exchange with CD_3OD showed an increase of one m.u. in M^+ confirming the presence of one exchangeable proton (OH group).

The ^1H-NMR spectrum ($CDCl_3$, 300 MHz) showed a three-proton double doublet at δ1.45 ($J_{18,19}$ = 7.2 Hz, $J_{18,21}$ = 1.9 Hz)

which was assigned to the methyl group of the ethylidine side chain. The C-19 olefinic proton appeared as a split quartet at δ 5.42 ($J_{19,18}$ = 7.2, $J_{19,15}$ ~ $J_{19,21}$ = 1Hz). Two singlets at δ2.69 and 3.51 were assigned to the N-CH_3 and carbomethoxy methyl protons respectively. The aromatic region showed the presence of four signals each integrating for one-proton, corresponding to the four aromatic protons of the dihydroindole nucleus.

Two dimensional NMR experiments (COSY-45, NOESY) were carried out to verify the assignments. The NOESY spectrum established the relative stereochemistry at various asymmetric centres and suggested the "E" configuration for the 19,20 double bond. The ^{13}C-chemical shifts are presented around structure 32.

On the basis of the above spectroscopic studies, structure 32 was assigned to strictaminolamine.

(D) NEW INDOLE ALKALOIDS FROM ALSTONIA SCHOLARIS

Alstonia scholaris (*Apocynaceae*) locally known as "chaliyum" is a large evergreen tree. The extract of the plant has been used in the indigenous system of medicine for the treatment of various diseases[69,70]. The alcoholic extract of the stem bark showed anti-cancer activity in HS_1 human sarcoma[71,72] and also exhibited significant antimicrobial activity[73]. The ethanolic extract of the leaves of *A. scholaris* has afforded the following new alkaloids.

Alstonamine (33)[74]

The UV spectrum (MeOH) of alstonamine showed absorptions at 222, 283 and 290 nm, characteristic of the indole chromophore. The IR spectrum ($CHCl_3$) showed absorptions at 3300 cm^{-1} (N-H) and 1725 cm^{-1} (ester C=O).

The HRMS showed the molecular ion peak at m/z 338.1632, corresponding to the molecular formula $C_{20}H_{22}N_2O_3$, indicating

eleven double bond equivalents in the molecule. Other significant peaks were observed at m/z 307, 251, 206, 157, 170 and 122.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) was very similar to that reported for vallesamine[75]. The main difference was the absence of signals for the methyl protons and the presence of two doublets centered at δ4.50 ($J_{18\alpha,18\beta}$ = 14.2 Hz, $J_{18\alpha,19}$ = 5.4 Hz) and at δ4.21 ($J_{18\alpha,18\beta}$ = 14.2 Hz, $J_{18\beta,19}$ = 3.4 Hz) which were assigned to the 18 α and β protons respectively. The ester methyl protons resonated as a 3H singlet at δ3.88 while the olefinic proton resonated at δ 5.53. The close resemblance of the ^{1}H-NMR signals of alstonamine with those of vallesamine, and the absence of the 18-methyl protons indicated that the C-18 carbon had undergone cyclization with the C-17 hydroxyl group to generate a new 7-membered ring in alstonamine 33.

In order to confirm the assignments in the ^{1}H-NMR spectrum a comprehensive series of homodecoupling experiments were carried out. The chemical shifts, coupling constants and ^{1}H-^{1}H coupling were confirmed by recording the 2D, COSY-45 and 2D J-resolved spectra. The ^{13}C-NMR assignments are presented around structure 33.

On the basis of above spectroscopic studies structure 33 was assigned to alstonamine.

Scholaricine (34)[76]

The UV spectrum (MeOH) of scholaricine showed absorptions at 210, 235, 285 and 335 nm, characteristic of an anilinoacrylate chromophore. The IR spectrum ($CHCl_3$) gave absorptions at 3500 cm^{-1} (O-H), 3400 cm^{-1} (N-H) and 1660 cm^{-1} (α, β -unsaturated C=O).

The HRMS afforded the molecular ion peak at 356.1736 corresponding to the molecular formula $C_{20}H_{24}N_2O_4$, indicating ten double bond equivalents in the molecule. The peak at m/z

257.1299 ($C_{15}H_{17}N_2O_2$) suggested the loss of 99 m.u., which corresponds to the cleavage of a fragment bearing the conjugated ester group.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed a three-proton singlet at δ3.83 which is assigned to the ester methyl protons. The 18-methyl protons afforded a doublet at δ1.12 ($J_{18,19}$ = 6.0 Hz) suggesting the presence of O-CH-CH_3 moiety as in scholarine[77]. Integration of the aromatic region showed the presence of only three protons, which indicated the existence of a substituent in the benzene ring. The aromatic protons gave a complex ABC type multiplets in the region δ6.63 to δ7.12. This suggested that the OH group was present at C-9 or C-12, as location of the OH group at C-10 or C-11 would have afforded a readily recognizable AB pattern. The ^{13}C-NMR assignments are presented around structure 34.

On the basis of above spectroscopic studies, structure 34 was assigned to scholaricine.

19,20-Z-Vallesamine (35)[78]

The UV spectrum (MeOH) of this new alkaloid was found to be characteristic for the indole chromophore, showing absorptions at 225, 275, 282 and 293 nm. The IR spectrum ($CHCl_3$) showed absorptions at 3300 cm^{-1} (N-H) and 1725 cm^{-1} (ester C=O).

The HRMS showed the molecular ion at m/z 340.1947, corresponding to the molecular formula $C_{20}H_{24}N_2O_3$, indicating ten double bond equivalents in the molecule. Other significant peaks were observed at m/z 208, 143 and 122. The mass fragmentation pattern was identical to that reported for vallesamine[75].

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed a doublet at δ 1.69 ($J_{18,19}$ = 6.4 Hz) for the ethylidine methyl group while the C-19 olefinic proton resonated at δ5.52 as a quartet ($J_{19,18}$ = 6.4 Hz). The ester methyl protons appeared at δ3.70 as a singlet. The C-6α and β protons appeared as a doublet each at δ4.93 and δ4.05 (J

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= 16.4 Hz), the downfield chemical shift reflecting the α-nitrogen function.

The ^{13}C-NMR spectrum ($CDCl_3$, 75MHz) showed 20 carbon resonaces. The multiplicity of each carbon atom was determined by using DEPT experiments. The experiments revealed the presence of one methyl carbon, five methylene carbons and six methine carbons, in agreement with structure 35. The chemical shifts of 19,20-Z-vallesamine were similar to those reported in the literature for vallesamine[75]. The major difference appeared at C-19 and C-20 carbons which were shifted by 3.32 ppm downfield and δ4.62 ppm upfield respectively, thereby indicating a change in the stereochemistry at the 19,20 double bond. The ^{13}C-NMR shifts are presented around structure 35. The NOE difference measurements also showed that the 19,20 double bond has "Z" configuration.

On the basis of the above spectral studies, structure 35 was assigned to the substance (19,20-Z-vallesamine).

(E) NEW INDOLE ALKALOIDS FROM ERVATAMIA CORONARIA

Ervatamia coronaria Stapf. is widely distributed in tropical countires as a garden plant. The plant has found wide use in the indigenous system of medicine for the treatment of various diseases[79]. The alkaloids from the plant caused temporary leukopenia in rats[80]. The ethanolic extract of the dried leaves of *Ervatamia coronaria* have afforded the following new indole alkaloids.

Ervaticine (36)[81]

The UV spectrum (MeOH) of this new alkaloid showed absorptions at 235 and 312 nm, indicating the presence of a 2-acyl indole chromophore. The IR spectrum (KBr) indicated the presence of a carbonyl group at 1640 cm^{-1}. The HRMS afforded molecular ion peak at m/z 266.1412 corresponding to the molecular formula $C_{17}H_{18}N_2O$, indicating ten double bond equivalents in the

molecule. Linked scan measurements of the metastable transitions were also carried out to verify the ion fragmentation pathway.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) of ervaticine was strikingly similar to that of vallesamine[75]. The C-18 methyl group appeared as a doublet at δ1.52 ($J_{18,19}$ = 6.9 Hz), while the C-19 olefinic proton resonated at δ5.49 ($J_{19,18}$ = 6.9 Hz). A doublet observed at δ3.98 ($J_{15,14}$ = 6.0 Hz) was assigned to the C-15 proton. The downfield shift for the C-15 proton was accounted for by the presence of the adjacent carbonyl group. The NH proton resonated as a broad singlet at δ8.92. The pattern of protons in the aromatic region was typical of an unsubstituted indole ring.

All these assignments and inter-relationships were confirmed by homonuclear decoupling as well as by 2D J-resolved and 2D NMR (COSY-45) experiments.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) showed various diagnostic carbon chemical shifts. The C-18 methyl carbon appeared at δ12.75. The signal at δ44.2 was assigned to C-15 which was located at the α-position of the carbonyl group. The signal due to the C-19 olefinic carbon atom appeared at δ126.70.

The presence of the 2-acyl indole moiety was confirmed by the reduction of ervaticine with sodium borohydride in methanol. The stereochemistry at some key centres of ervaticine was established by carrying out NOE difference measurements. These served to establish "Z" configuration of ethylidine side chain.

In the light of above spectroscopic evidences, structure 36 was assigned to ervaticine.

Ervatinine (37)[82]

This new alkaloid exhibited absorptions at 205, 227 ad 300 nm in its UV spectrum which were consistent with the presence of the indole nucleus. The IR spectrum ($CHCl_3$) showed absorptions at

3500 cm^{-1} (O-H), 3425 cm^{-1} (N-H), 2920 cm^{-1} (C-H), 1220 cm^{-1} (C-O-C, epoxide) and 1690 cm^{-1} (amide carbonyl group).

The HRMS afforded the molecular ion peak at m/z 326.1626 corresponding to the molecular formula $C_{19}H_{22}N_2O_3$, indicating ten double bond equivalents in the molecule. The peaks at m/z 152.1073 ($C_9H_{14}NO$), 140.1077 ($C_8H_{14}NO$) and 124.0810 ($C_7H_{10}NO$) revealed that the piperidine moiety bears only one oxygen atom. The fragments at m/z 279, 226 and 175 indicated that the carbonyl group of the amide function could be attached to C-5. Attempted reduction with sodium borohydride failed to afford any reduced product, supporting the presence of an epoxide group in the piperidine ring.

The ^{1}H-NMR spectrum ($CDCl_3$, 100 MHz) showed a triplet at δ0.81 ($J_{18,19}$ = 7.0 Hz) for the methyl protons and a quartet at δ1.12 ($J_{19,18}$ = 7.0 Hz) was attributed to the methylene protons of the ethyl group. A doublet at δ2.80 ($J_{15,14}$ = 4.5 Hz) and a multiplet at δ3.20 gave support to the presence of an epoxide group. These signals were assigned to the C-15 and C-14 protons respectively. The NH proton resonated as a broad singlet at δ 8.25.

On the basis of these spectroscopic data, structure 37 was assigned to ervatinine.

Hyderabadine (38)[83]

The compound afforded typically indolic UV spectrum (MeOH) showing absorptions at 229 and 284 nm. The IR spectrum ($CHCl_3$) afforded absorptions at 3300 cm^{-1} (N-H) and 2920-2850 cm^{-1} (C-H), but did not show any absorptions in the carbonyl region.

The HRMS afforded the molecular ion peak at m/z 340.2152, consistent with the molecular formula $C_{21}H_{28}N_2O_2$, indicating nine double bond equivalents in the molecule. Other prominent peaks

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appeared at m/z 311, 295, 265, 225, 183, 182, 157, 156, 152, 144, 143, 110 and 108.

The ^{1}H-NMR spectrum ($CDCl_3$, 100 MHz) showed the presence of a triplet centred at δ1,21 (J = 7.0 Hz) and a quartet centred at δ3.39 (J = 7.0 Hz) which were assigned to the methyl and methylene protons of an ethoxy group. A downfield doublet centred at δ 4.52 ($J_{15,14}$ = 7.0 Hz) was assigned to the C-15 proton. A complex multiplet in the region δ3.73-3.93 was ascribed to the oxymethylene protons at the C-18. A broad signal at δ8.25 was assigned to the indolic N-H group. The ^{13}C-NMR shift assignments are presented around structure 38.

In consideration of the data presented above, structure 38 was proposed for hyderabadine.

Lahoricine (39)[84]

The UV spectrum (MeOH) of this new alkaloid showed absorptions at 220 and 260 nm, indicating the presence of an indolenine chromophore. The IR spectrum ($CHCl_3$) did not show the presence of olefinic or carbonyl groups but indicated the presence of an ether linkage (absorption at 1150 cm^{-1}).

The HRMS showed the molecular ion peak at m/z 294.1719, corresponding to the molecular formula $C_{19}H_{22}N_2O$, indicating ten double bond equivalents in the molecule. The peaks at m/z 279 and 277 were attributed to the loss of methyl and hydroxyl group from the molecular ion respectively. The failure of attempted acetylation supported the presence of the oxygen atom as an ether linkage.

The ^{1}H-NMR spectrum ($CDCl_3$, 100 MHz) showed a three-proton doublet at δ0.93 ($J_{18,19}$ = 7.0 Hz) assigned to the C-18 methyl protons suggesting that it is adjacent to the carbon directly linked to the oxygen atom. The C-19 proton was observed as a multiplet centred at δ3.51. Another downfield multiplet at δ3.17 was assigned to the C-17 methylene protons. A downfield double

doublet centred at δ3.87 ($J_{3,14}$ = 3.0 Hz, $J_{3,14'}$ = 8.0 Hz) was attributed to the C-3 proton.

In view of the above spectroscopic studies, structure 39 was proposed for lahoricine.

Mehranine (40)[85]

This new alkaloid showed UV spectrum (MeOH) typical of the dihydroindole chromophore, showing absorptions at 209, 257 and 305 nm. The IR spectrum ($CHCl_3$) indicated the presence of N-CH_3 group (2800 cm^{-1}) and an epoxide group (1250 cm^{-1}).

The HRMS showed the molecular ion peak at m/z 310.2056, corresponding to the molecular formula $C_{20}H_{26}N_2O$, indicating nine double bond equivalents in the molecule. Since the IR spectrum did not show any absorptions in the carbonyl region and since attempted acetylation failed to give any acetylated product, it appear plausible that the oxygen atom was present as an epoxide linkage. The fragment ion at m/z 293.2046, attributed to the loss of hydroxyl radical, indicated the presence of an epoxide group in the molecule.

The ^{1}H-NMR spectrum ($CDCl_3$, 100 MHz) showed the presence of a ethyl group since it showed a triplet at δ0.81 ($J_{18,19}$ = 7.0 Hz) for the C-18 methyl protons, while the C-19 methylene protons were observed at δ1.27 ($J_{19,18}$ = 7.0 Hz) as a quartet. A three-proton singlet at δ2.75 indicated the presence of the N-methyl group. A doublet at δ2.96 ($J_{15,14}$ = 4.1 Hz) was assigned to the C-15 proton. The C-2 proton resonated as a double doublet centred at δ 3.58 ($J_{2,16\alpha}$ = 12 Hz, $J_{2,16\beta}$ = 6 Hz) while a singlet at δ2.25 was assigned to the C-21 proton. The aromatic protons appeared as a complex multiplet in the region of δ7.00-7.50.

In view of the aove spectroscopic studies, structure 40 has been proposed for mehranine.

Stapfinine (41)[86]

This new alkaloid afforded a typical UV spectrum (MeOH) showing absorptions at 222, 275 and 292 nm, characteristic for the indole chromophore. The IR spectrum ($CHCl_3$) showed absorptions at 3450 cm^{-1} indicating the presence of a hydroxyl group in the structure. Absorptions at 1460, 1360 and 1240 cm^{-1} were indicative of the presence of an epoxide or ether linkage.

The HRMS showed the molecular ion peak at m/z 321.1821 corresponding to the molecular formula $C_{19}H_{24}N_2O_2$, indicating nine double bond equivalents. The fragment observed at m/z 294.1728 ($C_{19}H_{22}N_2O$) due to the loss of 18 m.u., indicated the presence of a hydroxyl group in the molecule. Similarly, the fragments at m/z 138.0917 ($C_8H_{12}NO$) and 124.0761 ($C_7H_{10}NO$) demonstrated that only one oxygen atom is present on the piperidine ring.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed a 3H triplet at δ0.74 ($J_{18,19}$ = 7.4 Hz) and a quartet at δ1.22 ($J_{19,18}$ = 7.4 Hz) for the methyl and methylene protons of the ethyl group respectively. The C-15H resonated as a doublet at δ3.25 ($J_{15,14}$ = 3.0 Hz) and the C-14H appeared at δ2.90 as a multiplet. The rather upfield chemical shifts for the C-14 and C-15 protons indicated that the epoxide possessed a β-configuration[82]. A broad singlet at δ7.73 was assigned to the NH protons. The presence of four proton signals in the aromatic region suggested the lack of substitution on the indole chromophore. These assignments were confirmed by homo-decoupling experiments, 2D NMR (COSY-45, 2D J-resolved) experiments.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) was also in agreement with the proposed structure 41. The multiplicity assignments were made by the DEPT pulse sequence. The signal at δ68.90 was assigned to C-5 bearing the OH group. The C-14 and C-15 methine carbons were observed at δ53.10 and δ58.60 respectively.

All the other carbons displayed signals at expected values and the ^{13}C-NMR assignments are presented around structure 41.

On the basis of this spectral data, structure 41 is proposed for stapfinine.

(F) NEW INDOLE ALKALOIDS FROM PETCHIA CEYLANICA

Petchia ceylanica Wight is an evergreen herb, indigenous to the lowlands of Sri Lanka. The alcoholic extract of the leaves and stems of *Petchia ceylanica* have resulted in the isolation of the following new alkaloids.

Ceylanicine (42)[87]

This new alkaloid was isolated as a light yellow-coloured amorphous solid from the extracts of the stem of *Petchia ceylanica*. The UV spectrum (MeOH) showed absorptions at 245, 276 and 355 nm, suggesting a dihydroindole skeleton with N-C-N linkage[88]. The IR spectrum (KBr) showed absorption at 3400^{-1}(O-H), 1720 cm^{-1} (ester C=O) and 1765 cm^{-1} (γ-lactone).

The HRMS showed the molecular ion peak at m/z 692.3499, corresponding to the molecular formula $C_{41}H_{48}N_4O_6$, indicating twenty double bond equivalents in the molecule. Other major peaks appeared at m/z 664, 524, 496 and 495. The mass fragmentation pattern was found to be almost identical to that of desmethylpeceyline[89].

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) exhibited four 1H singlets at δ7.78, 7.74, 6.71 and 6.65, which were assigned to the aromatic protons at the C-9′, C-12, C-9 and C-12′ respectively. This indicated a similar substitution pattern in ceylanicine as in other dimers isolated from *Petchia ceylanica*[90]. The ethylidine methyl protons appeared as a split double doublet at δ1.65 ($J_{18,19}$ = 6.8 Hz, $J_{18,21\beta}$ = 1.0 Hz), which showed vicinal coupling with the quartet at δ5.41 for C-19H ($J_{19,18}$ = 6.8 Hz). The E-stereochemistry of the

ethylidine side chain was established by NOE difference measurements. The R-configuration of the hydroxy group at C-19′ was established by Horeau's method[91]. The N-methyl singlets appeared at δ2.73 and δ2.61, while a 3H singlet at δ3.78 was assigned to the carbomethoxy methyl protons.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) indicated the presence of eleven methine, ten methylene, five methyl and thirteen quaternary cabron atoms in addition to an ester carbonyl and γ-lactone carbonyl group. The ^{1}H-NMR and ^{13}C-NMR data indicated the attachment of the two moieties to form the common unsymmetrically substituted dibenzofuran system. A downfield methine at δ78.69 for the C-2′ indicated β-configuration of the proton at this centre[92]. The ^{13}C-NMR shift assignments are presented on structure 42.

On the basis of these studies, structure 42 was proposed for ceylanicine.

Ceylanine (43)[87]

This new alkaloid was isolated as a pale-coloured pink amorphous solid. The UV spectrum (MeOH) showed absorptions at 265, 300 and 330 nm. The IR spectrum (KBr) showed absorptions at 3400 cm^{-1} (O-H), 1768 cm^{-1} (C=O of γ-lactone) and 1735 cm^{-1} (ester C=O).

The HRMS showed the molecular ion at m/z 722.3592 corresponding to the formula $C_{42}H_{50}N_4O_7$, indicating twenty double bond equivalents in the molecule. A prominent peak at m/z 694 ($C_{40}H_{46}N_4O_7$), corresponded to the loss of ethylene by a retro Diels-Alder cleavage of the ring D.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed a 3H singlet for the N-methyl protons at δ2.72, a 3H singlet for the ester methyl protons at δ3.64, two 3H singlets for the six methoxy protons at δ3.72 and δ3.82. The lack of splitting and the chemical

shifts of aromatic protons indicated that the substituents were present at the C-10,C-10′, C-11 and C-11′ positions. A three-proton doublet at δ1.59 ($J_{18,19}$ = 6.8 Hz) was assigned to the C-18 methyl while the 3H split doublet at δ1.18. A one-proton split doublet at δ4.68 ($J_{16',15'}$ = 6.0 Hz) was characteristic for the C-16′H. The upfield chemical shift for the C-16′H established it to be β-oriented[93].

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) exhibited eleven methine, ten methylene and six methyl carbons, the multiplicity was established by DEPT experiments. The ^{13}C-NMR shift assignments are shown around structure 43. The NOE difference measurements established the E-stereochemistry of the ethylidine side chain. The R-configuration of the hydroxyl group at the C-19′ was established by Horeau's method[91].

On the basis of the above data structure 43 was assigned to ceylanine.

Desmethylpeceyline (44)[89]

A new dimeric indole alkaloid desmethylpeceyline was isolated from the leaves of *Petchia ceylanica*. The UV spectrum (MeOH) showed absorptions at 204, 245, 272 and 347 nm, consistent with the presence of a chromophore comprising an indoline system with another nitrogen atom β to the indoline nitrogen[88]. The IR spectrum ($CHCl_3$) showed absorptions at 3400 cm^{-1} (N-H) and 1738 cm^{-1} (ester C=O).

The HRMS afforded M^+ at m/z 690.3465, corresponding to the formula $C_{41}H_{46}N_4O_6$, indicating twenty-one double bond equivalents in the molecule. The mass fragmentation follows a path similar to that of corymine[94].

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed four aromatic proton signals as singlets which appeared at δ6.51, δ6.67, δ7.40 and δ7.41 assigned to C-12′, C-12, C-9 and C-9′ protons

respectively, suggesting that there were two aromatic protons in each moiety with a *para* disposition to one another. The C-19 olefinic proton resonated as a quartet at δ5.12 ($J_{19,18}$ = 6.7 Hz). The two 3H singlets at δ3.83 and δ3.84 was assigned to the two methoxy groups. The two C-methyl doublets appeared at δ 1.31 ($J_{18',19'}$ = 6.0 Hz) and δ1.69 ($J_{18,19}$ = 6.7 Hz) corresponding to the C-18′ and C-18 methyl protons respectively. The C-19′ oxymethine proton resonated as a quartet at δ3.09 ($J_{19',18'}$ = 6.0 Hz) while the N-methyl protons appeared at δ 2.75.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) showed the presence of the ethylidine, O-methyl and N-methy groups. The ^{13}C-NMR shift assignments are presented around structure 44. The NOE difference measurements established that the vinocrine moiety of desmethylpeceyline bears the N-methyl group while the other moiety is demethylvincorine oxide.

On the basis of the above spectroscopic data, structure 44 was assigned to desmethylpeceyline.

(19R)-Epimisiline (45)[91]

The UV spectrum (MeOH) of this new alkaloid was characteristic of an anilinoacrylate chromophore showing absorptions at 328, 297 and 205 nm. The IR spectrum ($CHCl_3$) gave absorptions at 3500 cm^{-1} (O-H), 3350 cm^{-1} (N-H) and 1680 cm^{-1} (α,β - unsaturated C=O).

The HRMS of the alkaloid showed the molecular ion peak at m/z 368.1741, corresponding to the molecular formula $C_{21}H_{24}N_2O_4$, indicating eleven double bond equivalents in the molecule. Its mass fragmentation pattern indicated the presence of an aspidosperma skeleton.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed a doublet at δ1.15 ($J_{18,19}$ = 7.0 Hz) which was assigned to the C-18 methyl protons, its chemical shift being consistent with the presence of a -

CH (OH)CH_3 moiety, or scholaricine[76]. The C-19 methine proton geminal to the hydroxyl group resonated as a multiplet centred at δ3.35. The C-3α proton resonated at δ2.90 as a multiplet while a double doublet at δ3.52 ($J_{3\beta,3\alpha}$ = 12.7 Hz, $J_{3\beta,4\alpha}$ = 5.4 Hz) was assigned to the C-3β proton. A three -proton singlet at δ 3.79 was assigned to the ester methyl group. The aromatic protons appeared as multiplets at δ6.79-7.22. The NH proton appeared as a singlet at δ 8.88.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) of (19R)-epimisiline showed 21 carbon resonances. The multiplicity assignments were made on the basis of polarization transfer experiments (DEPT).

The ^{13}C-NMR shifts are presented around structure 45. The 2D NMR experiments (COSY-45 and 2D J-resolved) were also carried out to determine the ^{1}H-^{1}H coupling interactions and multiplicities of the proton signals respectively. On the basis of the above spectral data, structure 45 was assigned to (19R)-epimisiline.

(19S)-Epimisiline (46)[91]

The UV spectrum (MeOH) of (19S)-epimisiline was characteristic of an anilino-acrylate chromophore showing absorptions at 226, 297 and 328 nm. The IR spectrum ($CHCl_3$) showed intense absorptions at 3500 cm^{-1} (O-H), 3350 cm^{-1} (N-H) and 1680 cm^{-1} (α,β - unsaturated C=O).

The HRMS afforded the molecular ion peak at m/z 368.1743, corresponding to the molecular formula $C_{21}H_{24}N_2O_4$, indicating ten double bond equivalents in the molecule. The mass fragmentation pattern was similar to that of (19R) -epimisiline[91].

The 1H-NMR spectrum ($CDCl_3$, 300 MHz) corresponded closely to that of (19R)-epimisiline[91], the major differences appearing at the chemical shifts for C-19H which appeared at δ3.59, 0.24 ppm downfield than the chemical shift of C-19H in (19R)-epimisi-

line. This suggested that (19S)-epimisiline was the C-19 epimer of (19R)-epimisiline.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) of (19S)-epimisiline was also very similar to that of (19R)-epimisiline[91]. Particularly revealing was the fact that the chemical shifts of C-21 in both compounds was virtually identical. This supported the conclusion that the epoxide function was in the β-configuration in (19S)-epimisiline[91], since in the α-configuration C-21 would have been expected to resonate downfield. These results indicate that the only point of structural difference between (19R)-epimisiline (45) and (19S)-epimisiline lay in the stereochemistry of the C-19 hydroxyl group. The ^{13}C-NMR chemical shifts are presented around structure 46. The 2D NMR experiments (COSY-45 and 2D J-resolved) also served to establish structure 46 for (19S)-epimisiline.

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